

BIODEGRADATION OF AIRCRAFT DEICING FLUID  
COMPONENTS IN SOIL

Baron W. Burke, B.S.  
Captain, USAF

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THESIS

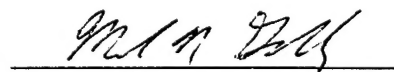
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March 1999

Presented to the Faculty of the School of Engineering  
of the Air Force Institute of Technology  
Air University  
In Partial Fulfillment of the  
Requirements for the Degree of  
Masters of Science in Engineering and Environmental Management

  
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## Abstract

Aircraft de-icing fluids (ADFs) are used by commercial and military agencies to ensure safe aircraft operations. Disposal of these spent fluids can pose environmental concerns. Propylene Glycol (PG) is one of the main glycol materials used in ADF, and its biodegradability in various media has been very well documented. However, its high biochemical oxygen demand can pose a severe risk to treatment facilities and water bodies around an airfield. Another unknown is the environmental fate and biodegradability of individual additives in ADFs, such as wetting agents, thickeners, surfactants or corrosion inhibitors like tolyltriazole (TTA).

This research investigates the biodegradation activity of PG alone, TTA alone, and PG with TTA in an aerobic (high-clay) soil environment. This research effort used three test methods to measure the microbial response to these ADF chemical components. Automated respirometry indicated the behavior of the microbial activity through measured oxygen consumption and carbon dioxide production. High performance liquid chromatography (HPLC) was used to measure the residual TTA in soil after respirometry tests were completed. Toxicity tests, such as microbial colony population counts (MCPC) and agar well diffusion tests (AWDT), were used to measure the microbial response to these ADF chemical components.

This research was partitioned into two distinctive phases of investigation. Phase-one analyzed individual and combined ADF chemical components in uncontaminated soil. The presence of TTA, from 25 – 1,000 mg/kg, reduced the maximum respiration rate of 1,000 mg/kg PG alone; however, cumulative respiration over the two-week study

period was nearly the same. Respiration rates in soil exposed to only TTA were not significantly different from background rates.

HPLC analysis was performed after two-weeks of respirometry monitoring in phase-one research. The percentage of recovered TTA ranged from 49 – 56% and 79 – 86%, for 25 and 250 mg/kg TTA alone in soil, respectively. The percentage of recovered TTA ranged from 35 – 44% and 69 – 77%, for 25 and 250 mg/kg TTA with PG (1,000 mg/kg), respectively. The percentage of recovered TTA, with or without PG presence, indicated biodegradation and absorption of TTA within the soil environment. HPLC research was performed by Kellner's (1999) absorption/desorption of measurements of TTA with the same (high-clay) soil.

Toxicity tests were performed on microorganisms/soils from phase-one research. The MPCP indicated no measurable difference between microbial populations of uncontaminated soil versus treated soil with ADF chemical components. AWDT indicated no toxic effects from application of TTA solutions of 5,000 – 10,000 mg/L and PG solutions of 10,000 mg/L, individually and combined, upon microorganism within the test methods.

Phase-two research analyzed the re-application of ADF chemicals on acclimated soils from phase-one research. Specifically, oxygen consumption resulting from reapplication of 1,000 mg/kg PG on acclimated soil (PG 1,000 mg/kg) was compared to one-time application of 1,000 mg/kg PG on the uncontaminated soil. Maximum respiration rates were greater for the acclimated soil compared to the uncontaminated soil.

# BIODEGRADATION OF AIRCRAFT DEICING FLUID

## COMPONENTS IN SOIL

### I. Introduction

#### 1.1 Overview

Glycol based de-icing fluids are used at airport facilities worldwide to prevent snow and ice accumulation on aircraft and airfield surfaces. Glycol based de-icing fluid use ranges from approximately 95,000 L (25,000 gal)/y for a small military base to 5.7 million L (1.5 million gal)/y for a commercial airline [Strong-Gunderson *et al.*, 265]. Typically, a large aircraft will use 3,785 L (1,000 gal) of de-icing fluid [Mericas and Wagoner, 39]. There are two distinctive types of de-icing fluids used on aircraft. Aircraft de-icing fluid (ADF) is primarily used for immediate removal of snow and ice prior to aircraft takeoff. Aircraft de-icing/anti-icing fluid (ADAF) has a longer retention time on aircraft surfaces, thus allowing a longer hold time on the ground prior to takeoff. Both of the aircraft de-icing fluids (ADF and ADAF) have demonstrated their excellent reliability in maintaining safe aircraft operations [Mericas and Wagoner, 39-40]. In this thesis, the term aircraft de-icing fluids (ADFs) will refer to both ADF and ADAF.

The ratio of ADF concentrate to water typically ranges from 50:50 to 10:90 [Safferman *et al.*, 11] before application on the aircraft. This ratio depends on the ADF's manufacturer and weather conditions. ADFs concentrate is mainly glycol with some additives. Extensive studies have shown that glycols are readily degradable under many

environmental conditions. The main environmental concern lies with the high biochemical oxygen demand (BOD) placed upon receiving streams, water bodies, and wastewater treatment plants by the glycols.

Aircraft de-icing fluids also contain other essential additives that serve as corrosion inhibitors, thickeners, and surfactants [Hartwell *et al.*, 1375]. One specific pair of chemical isomers, 5(6)-Methyl-1H-Benzotriazole, are used as additives for corrosion protection [Cancilla *et al.*, 433-434]. Recently, studies by Cornell (1998) and Johnson (1997) investigated the effects on microbial degradation from combinations of tolyltriazole (TTA) and propylene glycol (PG) within a soil environment. The studies were performed in response to proposed "landfarm remediation" of spent ADFs. The results from the investigations were inconclusive. These inconclusive results suggest the need for further investigation. This research will expand our knowledge of tolyltriazole and propylene glycol effects on microbial degradation activity.

## **1.2 Specific Problem**

Aviation operations in cold weather regions require the use of ADFs to keep airfield and aircraft surfaces free from ice and snow. With passenger safety in mind, the Federal Aviation Administration enforces strict requirements for de-icing procedures [Mericas and Wagoner, 39]. After application of ADFs to aircraft or runway surfaces, a significant amount will be deposited upon the airfield. Typically 80% of the fluids are deposited on the ground due to spray drift, jet blast, and wind shearing during taxi and takeoff [Hartwell *et al.*, 1376]. The ADFs typically have two main routes to follow once deposited on the airfield. The ADFs can immediately become part of surface water



runoff, due to the frozen grounds' inability to absorb large amounts of runoff. Diluted ADFs can also be retained in snow pile deposits around the airfield until melting/run-off occurs [Transport Canada, 1985; MacDonald *et al.*, 10-13].

The glycol-based effluents (ADFs and water) eventually migrate into the environment where they might have detrimental effects. Diluted formulations and runoff at 1% deicer solution would have a BOD<sub>5</sub> of around 10,000 mg/L. Untreated raw domestic sewage has a BOD<sub>5</sub> of only 200 mg/L [Sills and Blakeslee, 1992]. The extremely large impact of de-icing fluids on water bodies has prompted pollution controls concerning this effluent. An airport group permit, which requires careful control and disposal with effluents, is issued under the Clean Water Act's Stormwater Regulations, specifically the National Pollutant Discharge Elimination System (NPDES) permit program [Oakley and Forrest, 52; Safferman *et al.*, 11].

The disposal of an ADF effluent can amount to an enormous cost due to the amount of dilution water required to meet treatment plant requirements. Restriction of 1 to 5% glycol concentration is the typical range that the treatment facilities will and can accept [Strong-Gunderson *et al.*, 326]. If glycol is not diluted to these levels, then a "shock load" or very high oxygen demand can occur within a wastewater treatment facility. This shock load can seriously affect the performance of the treatment plant [Metcalf and Eddy, 205].

The costs associated with disposal have prompted some recent investigation into recycling the spent fluids for resale back to manufacturers. In the 1990's, Denver's Stapleton Airport collected glycol solution and effectively sold the effluent when glycol concentrations were above 15% [Backer *et al.*, 58]. Airports considering recycling must

standardize their use of ethylene or propylene glycol because mixed streams of the two compounds have virtually no recycle value [Mericas and Wagoner, 48].

The other option of interest is the investigation for on-site treatment through the application of landfarm bioremediation. Therefore, a fundamental understanding of the interactions between the chemical components of ADFs in soils is crucial before landfarming application could ever become feasible.

### **1.3 Research Objectives**

The purpose of this research was to evaluate the biodegradation of propylene glycol with different levels of tolyltriazole in (high-clay) soil. The mixture and reapplication of these two ADF components were also varied to determine any effects upon soil microorganisms.

Respirometry was used to measure the consumption/uptake of oxygen and the production of carbon dioxide due to the degradation of propylene glycol and tolyltriazole. The microbe rich soil provided an aerobic system for observing the effects on microbial biodegradation from different combinations of the two chemicals. A Micro-Oxymax<sup>®</sup> "closed circuit" respirometer was used to monitor oxygen consumption and carbon dioxide production.

High performance liquid chromatography (HPLC) was used to analyze the residual amounts of tolyltriazole remaining in the soil once the respirometry experiments were complete. The HPLC data was not a complete representation of all biodegradation, due to chemical and physical process that could not be accounted for. However, it provided supplemental information to compare with the respirometry analysis. The

HPLC analysis also supported Kellner's (1999) thesis on absorption/desorption of tolyltriazole within the same (high-clay) soil.

Microbial colony plate counts (MCPC) and agar well diffusion tests (AWDT) were used to help determine whether the tolyltriazole present in different treatments induced microbial toxicity.

This investigation complements research performed on these two ADF components (Johnson, 1997; Cornell *et al.*, 1997). The respirometry research will address new areas of study, by using a larger variety of tolyltriazole treatments (25 – 1,000 mg/kg) with a fixed propylene glycol (1,000 mg/kg) treatment level, individually and combined in soil. Specific research are listed below:

1. Determine the influence on microbial degradation activity from either propylene glycol or tolyltriazole separately in uncontaminated soil environment.
2. Determine the combined influence on microbial degradation activity of tolyltriazole with propylene glycol in a uncontaminated soil environment.
3. Determine if there is any difference in microbial degradation activity when propylene glycol (1,000 mg/kg) is applied to uncontaminated soil/microorganism and preconditioned soil/microorganisms with propylene glycol.
4. Determine if varied combinations and concentrations of ADF chemical components of tolyltriazole and propylene glycol have a toxic effect upon microbial populations in soil.

#### **1.4 Scope**

A phased approach was used to accomplish the scope of this study. The first

phase tested the biodegradability of ADF chemical components (propylene glycol and tolyltriazole) at different concentrations and combinations in previously uncontaminated soil. The second phase of testing compared microbial activity of uncontaminated soil to the activity of ADF acclimated soil/microorganisms. The soils used/monitored in the phase-one studies were used in the phase-two as the acclimated soil/microorganisms.

Control of the test conditions and materials should limit variations in the investigation. Control of experimental conditions included; temperature, light, and moisture within the soil environments. Some constraints and assumptions on the scope of this research are as follows:

1. The same (high-clay) soil was used throughout all experiments.
2. Soil moisture was established at ~60% of field capacity (FC) prior to all respirometer experiments. As the respirometer supplies dry O<sub>2</sub> to the soil, there is a potential for that declining moisture content to reduce microbial metabolism. Long runs (over two weeks) were avoided to reduce this potential influence.
3. All propylene glycol applications on soil were held at 1,000 mg/kg.
4. Adequate nutrients (K, N, P) were present within the soil so as not to limit microbial activity (shown in the independent soil analysis, Appendix A).
5. Adequate aerobic conditions were assumed for all respirometer tests.
6. Uniform preparation techniques were maintained for all experimental runs.
7. Photo-degradation was considered negligible since soil in the respirometry experiments was kept in the dark.
8. Soil and chemicals were maintained in the dark and kept in cool conditions of 4°C to reduce the potential of chemical degradation between experimental runs.

9. Volatilization of chemicals was assumed negligible. This is assumed based on the chemical characteristics of propylene glycol and tolyltriazole.
10. Adequate numbers of microorganisms were assumed to exist in the soil. As this soil was collected in a natural environment, however it was not tested in any way. The assumptions appear reasonable.
11. Sorption/loss of chemicals to glass equipment used in experiments is assumed negligible. Kellner's (1999) results indicate some absorption of tolyltriazole in the (high-clay) soil. However, minimal loss occurs and is assumed negligible.

## **1.5 Summary**

This research investigated the aerobic microbial biodegradation potential of propylene glycol and tolyltriazole in a (high-clay) soil environment. Microbial respiration is a tool that can measure microbial activity within a soil environment under differing chemical combinations/treatments. HPLC analysis supported respirometry results. MCPC and AWDT are also tools for measuring toxicity effects from various chemical concentrations and mixtures. The results will support a better understanding of the biodegradation effects of two ADF components in a soil.

## **1.6 Terms Used in this Study**

Aerobic – Having molecular oxygen present; growing in the presence of oxygen.

Anaerobic – Living, active, or occurring in the absence of free oxygen.

Aircraft De-icing Fluid (ADF) - Used for the immediate removal of snow and ice from aircraft surfaces.

Aircraft De-icing/Anti-icing Fluid (ADAF) – Used for the immediate removal of snow and ice from aircraft surfaces, along with prevention of snow and ice build up on surfaces for a limited time.

Aircraft De-icing Fluid(s) (ADFs) – Refers to both ADF and ADAF for simplicity in the thesis discussion.

Biochemical Oxygen Demand (BOD) – The amount of molecular oxygen used by microorganisms in wastewater, effluents, and polluted waters for the biochemical degradation of organic material and the oxidation of inorganic material. BOD determination is an empirical test that uses standard laboratory procedures and is conducted over a specified time period, usually five days [Eaton *et al.*, 5-2].

Biodegradation – The microbial process of chemical breakdown of a substance into smaller products caused by microorganisms or their enzymes [Atlas and Bartha, 535].

Hydrophobic Organic Compound – Organic compounds with low solubility in aqueous solutions.

Hydrophilic Organic Compound – Organic compounds with high solubility in aqueous solutions.

Organic – Carbon containing compounds, typically containing carbon-carbon bonds [Brown *et al.*, G-11].

Oxidation – A process in which a substance loses one or more electrons [Brown *et al.*, G-11].

Metabolism – Chemical changes within living cells by which energy is provided for microbial growth and the necessary maintenance of cell life [McKane and Kandel, 9].

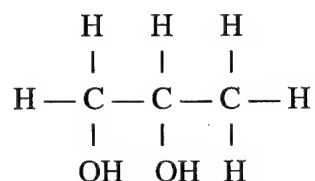
Microorganisms – Organisms that exist naturally in the environment such as bacteria, fungi, algae, protozoa, and viruses [Atlas and Bartha, 541].

Micro-Oymax<sup>®</sup> respirometer – An indirect closed loop respirometer designed to detect extremely low levels of oxygen uptake and carbon dioxide output for a variety of studies involving microorganisms, insects, plants, food, and chemical oxidation [Micro-Oymax<sup>®</sup> v6.03, Instruction Manual, 3].

Mineralization – The microbial breakdown of organic materials to inorganic materials brought about mainly by microorganisms [Atlas and Bartha, 541].

Propylene Glycol (PG) – Chemical used in ADF/ADAF; C<sub>3</sub>H<sub>8</sub>O<sub>2</sub>, See Figure 1-1 below for structure.

Figure 1-1  
Propylene Glycol, 1,2-Propanediol



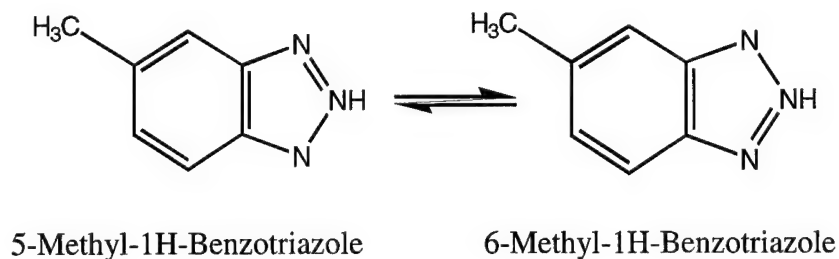
Respirometry – The measurement of the oxygen uptake and the carbon dioxide output associated with biological or chemical systems [Micro-Oymax<sup>®</sup> v6.03].

Respirometry Exchange Rate (RER) – The ratio of oxygen uptake to carbon dioxide output,  $O_2/CO_2$  [Micro-Oxymax<sup>®</sup> v6.03, Instruction Manual].

Statistical hypothesis – claim about the value of a single population characteristic, or about the values of several characteristics [Devore, 304].

Tolyltriazole (TTA) – Chemical used as a corrosion inhibitor in ADF/ADAF,  $C_7H_7N_3$ . There are two isomers for tolyltriazole. See Figure 1-2 below for structure [Cornell *et al.*, 1997].

Figure 1-2  
Tolyltriazole



Field Capacity (FC) – The maximum amount of water that an unsaturated zone of soil can hold against the pull of gravity [Fetter, 639].

Natural Attenuation – The oxidation or breakdown of a substance through natural processes.

Transformation – A reaction that occurs chemically or biologically by means of oxidation or reduction process.



## II. Literature Review

### 2.1 Background on Aircraft De-icing Fluids

Type I ADF is used as a de-icing fluid for aircraft surfaces, while type II ADAF is used as both a de-icing and anti-icing fluid that sticks to aircraft surfaces and inhibits subsequent ice formation during taxi and takeoff [Hartwell *et al.*, 1375]. Although the exact formulations of ADF/ADAFs are proprietary, the main components are glycol materials (90 – 99%) and a small amount of additives (1 – 10%) [SAE, 1992; Cornell, 2; Cancilla *et al.*, 430]. The mixture of concentrated ADF and water can typically be in the range of 50:50 to 10:90 [Safferman *et al.*, 11]. Another difference between ADF/ADAFs are the performance enhancements provided by the additives [Hartwell *et al.*, 1375].

The International Standards Organization (ISO) and Society of Automotive Engineers (SAE), specifically the division of Aircraft Maintenance Chemicals and Materials committee, helps to develop the specifications for commercial ADF/ADAF composition [Boluk and Levesque, 6]. These specifications are guidelines for the fluid application, viscosity, and metal corrosion inhibition qualities for aircraft application. The military specifications covering aircraft de-icing fluids is MIL-A-8243, which specifies two products. First, the military type I ADF, which is propylene glycol based. Second, the military type II ADAF, which is ethylene glycol based (three parts ethylene glycol and one part propylene glycol [Environmental Department of the Naval Facilities Engineering Service Center, 1998].

A directive issued on March 31, 1992 from Brigadier General James E. McCarthy, the Air Force Civil Engineer, placed an immediate USAF-wide prohibition on

the use of ethylene glycol upon all airfield operations. This banning of the ethylene glycol based ADF caused the Air Force to specify propylene glycol based solution to be used throughout all Air Force bases [HQ Air Force Center for Environmental Excellence, 1995].

Type I ADF (commercial) can be a mixture of glycol (ethylene glycol, diethylene glycol, and/or propylene glycol) along with corrosion inhibitors, either 1H-Benzotriazole (BTA) or 5(6)-Methyl-1H-Benzotriazole, common name tolyltriazole (TTA). TTA is used in more ADF formulations than BTA [Cornell, 1997]. The other additives are flame-retardants and surfactants (wetting agents/detergents) made to keep chemicals within the solution. The fluid is typically clear, orange in color [Bausmith, 3; Cancilla *et al.*, 430; Hartwell *et al.*, 1995].

The type II ADAF (commercial) can be a mixture of glycol (ethylene glycol, diethylene glycol, and/or propylene glycol) along with corrosion inhibitors, flame-retardants, and surfactants (wetting agents/detergents), plus thickeners that cause adhesion to the aircraft surface. These thickening agents require a different suite of corrosion inhibitors and surfactants than those used in type I fluids. Typically, the adhesion additive is a polymer, which is neutral and anionic. The fluid is typically clear, pink in color [Bausmith, 3; Cancilla *et al.*, 430; Hartwell *et al.*, 1995].

### **2.1.1 Environmental Fate of Spent Aircraft De-icing Fluids**

Of the ADFs applied, it is estimated that only 16% of the fluid remains on the aircraft surfaces. The amount that falls off the plane is usually collected at the application point using a sump style collection pad. However, the fluids that are retained

do eventually leave the aircraft at some point. An estimated 49% falls on the ground and 35% is lost to wind [Transport Canada, 1988].

The transport of used ADFs that have fallen to the ground is not always direct and simple. ADFs can persist even after the last application of ADFs within a season. An estimated 30% of the de-icer fluid applied will be stored in snow piles to be released during spring rains and snowmelt [Transport Canada, 1988].

### **2.1.2 Regulations Concerning Spent Aircraft De-icing Fluids**

The Environmental Protection Agency (EPA) Storm Water Discharge regulations went into effect on December 17, 1990. These regulations placed storm water under the National Pollution Discharge Elimination System (NPDES) permit program. Under the 1990 regulations, the NPDES permit program now covers effluents previously considered non-point sources [Oakley and Forrest, 1991]. These storm water discharges are associated with industrial activities, including operations such as airports (commercial and military). These industrial activities that result in direct storm water discharge into waters of the United States and storm water discharge through municipal storm sewers are required to obtain NPDES permits from the EPA [Leiter and Funderbunk Jr., 22-23].

The EPA delegated administration of the NPDES program to local state-regulatory agencies. This allowed for some state-to-state difference in handling of the permitting program [Boyd, 1991]. The ultimate outcome was a requirement for proper treatment of stormwater runoff. The water can be treated on site, discharged to publicly owned treatment works, or perhaps recycled [Mericas and Wagoner, 39].

In response to the options available for storm water disposal, new airports began

more active management of these spent ADFs. Newer airports began designing collection and recycling systems, while existing airfields altered their collection and disposal techniques to meet the regulations. This has also led to a renewed interest in handling of these fluids on site.

## 2.2 Aircraft De-icing Fluids Chemical Components

### 2.2.1 Properties of Propylene Glycol

The structure of propylene glycol is composed of two OH (alcohol) groups attached to the 1 and 2 carbons (See Figure 1-1). Table 2-1 summarizes the properties of propylene glycol.

Table 2-1  
Chemical Characteristics of Propylene Glycol

| 1,2-Propanediol (Propylene Glycol) Characteristics      | Result                | Reference            |
|---|-----------------------|----------------------|
| Boiling Point (°C) at 760 mm Hg                         | 188.2                 | Sax and Lewis (1998) |
| Freezing Point (°C) at 760 mm Hg                        | -59                   | Sax and Lewis (1998) |
| Vapor Pressure (mm HG) at 20°C                          | 0.08                  | Sax and Lewis (1998) |
| Solubility in Water                                     | hydroscopic           | Sax and Lewis (1998) |
| Octanol/Water Partition Coefficient ( $K_{ow}$ )        | $3.89 \times 10^{-2}$ | Miller (1979)        |
| Organic Carbon/Water Partition Coefficient ( $K_{oc}$ ) | $2.4 \times 10^{-2}$  | Miller (1979)        |

### 2.2.2 Properties of Tolyltriazole

The isomers of 5(6)-methyl-1H-benzotriazole, common name “tolyltriazole” (See Figure 1-2), having the methyl group substituted at one of the other positions on the aromatic ring [Cancilla *et al.*, 1996]. The properties of the benzo-ring structure are assumed to make the tolyltriazole compound difficult to degrade. Table 2-2 summarizes

the properties of tolyltriazole.

Table 2-2  
Chemical Characteristics of Tolyltriazole

| 5(6)-Methyl-1H-Benzotriazole (Tolyltriazole) Characteristics | Result                | Reference              |
|--|-----------------------|------------------------|
| Boiling Point (°C) at 760 mm Hg                              | 160                   | PMC Specialties (1996) |
| Freezing Point (°C) at 760 mm Hg                             | 76-87                 | PMC Specialties (1996) |
| Vapor Pressure (mm HG) at 20°C                               | 0.03                  | PMC Specialties (1996) |
| Solubility in Water  | hydrophobic           | PMC Specialties (1996) |
| Octanol/Water Partition Coefficient ( $K_{ow}$ )             | $3.35 \times 10^{-1}$ | Lyman (1982)           |

### 2.2.3 Toxicity/Hazards of Propylene Glycol

Literature indicates that pure glycol may be acutely toxic to aquatic life at sufficiently high concentrations. Propylene glycol is not known to be a carcinogen or teratogen [Mallinckrodt, 1997]. The toxicity level of propylene glycol has been established through several studies. Studies reviewed by MacDonald *et al.* (1992) on aquatic organisms (juvenile trout) revealed a median  $LC_{50} > 50,000$  mg/L for a 24 hour period [Majewski *et al.*, 1978]. Bridie *et al.* (1979) conducted bioassays on goldfish, which suggested propylene glycol was not acutely toxic at levels below 5,000 mg/L.

Exposure hazards to propylene glycol (pure aqueous) include eye, nose, and throat irritation. High levels become objectionable because of the chemical's odor [Mallinckrodt, 1997].

### 2.2.4 Toxicity/Hazards of Tolyltriazole

Tolyltriazole is not considered a carcinogen and chronic toxicity data is not available. Research by PMC Specialties Group, indicates a moderate toxicity to aquatic

organisms from the tolyltriazole isomers on *Lepomis machorochirus* (31 mg/L 96 hr, LC<sub>50</sub>) and *Daphnia magna* (74 mg/L 48 hr, LC<sub>50</sub>).

According to the material safety data sheet, tolyltriazole presents moderate risks to health by inhalation, ingestion, or skin absorption [PMC Specialties, 1996]. Thus, appropriate procedures are recommended to prevent opportunities from direct contact with the skin or eyes and to prevent inhalation.

### **2.3 Biodegradation**

The biodegradation process can be influenced by many different conditions. Physical, chemical, and biological conditions directly affect the microorganisms' ability to metabolize a carbon compound into food or energy.

The health and concentration of microbial populations has been directly related to natural or manmade conditions. The competitive environment of nature encourages robust and hardy populations of microbes [Atlas and Bartha, 53]. Other important factors affecting microorganism health and activity are availability of moisture and inorganic nutrients.

Soil microbes require essential mineral nutrients along with a carbon source for unhampered metabolic processes to occur. These essential nutrients for healthy cells are: hydrogen, nitrogen, phosphorus, and sulfur. Hydrogen and oxygen, along with carbon, are essential for synthesis of most organic compounds. Phosphorus is needed for adenosine triphosphate (ATP) and nucleic acids, sulfur for protein, and nitrogen for nucleic acids and protein [McKane and Kandel, 106].

Aerobic metabolism requires oxygen as an electron acceptor for use in the consumption of carbon sources. The pH and temperature of the environmental media can directly influence the health and optimal rate of degradation for microbes.

The benefit of biodegradation is the conversion of contaminants into more environmentally safe compounds, such as carbon dioxide and water.

### **2.3.1 Effect of Temperature on Biodegradation**

Temperature affects microbial degradation of carbon within a soil environment. The activity of aerobic microorganisms indigenous to soil is highest at temperatures of 20 - 30°C [Atlas and Bartha, 218].

Preliminary studies by Klecka *et al.* (1993) indicated that there was an increased biodegradation rate of three different glycol (ethylene, diethylene, and propylene glycol) and five different brands of ADFs, with an increase in soil temperature. The three glycols degradation rates were similar, ranging from 19.7 to 27.0 mg/kg soil per day to 66.3 to 93.3 mg/kg soil per day for samples at 8°C and 25°C, respectively (Klecka *et al.*, 292). This indicated a 3.4 faster rate of microbial degradation for the difference in temperature. Research by Rice *et al.* (1997) indicated a similar relationship between the soil temperature and ethylene glycol mineralization rate.

### **2.3.2 Effects of pH on Biodegradation**

The pH varies in different layers of soil. The upper layer is typically more aerobic and saturated from rainfall than lower layers. The result is that there is more

acidity in the upper layers [Metting, 1993]. Most bacteria and fungi tolerate alkaline pH up to 9.0 but have a pH optima near neutrality [Atlas and Bartha, 234].

### **2.3.3 Effects of Soil Moisture on Biodegradation**

Optimal conditions for activity of aerobic soil microorganisms occurs between 50 and 70% of the water holding capacity of the soil. A higher water content, although not inhibitory by itself, starts to interfere with oxygen availability [Atlas and Bartha, 229].

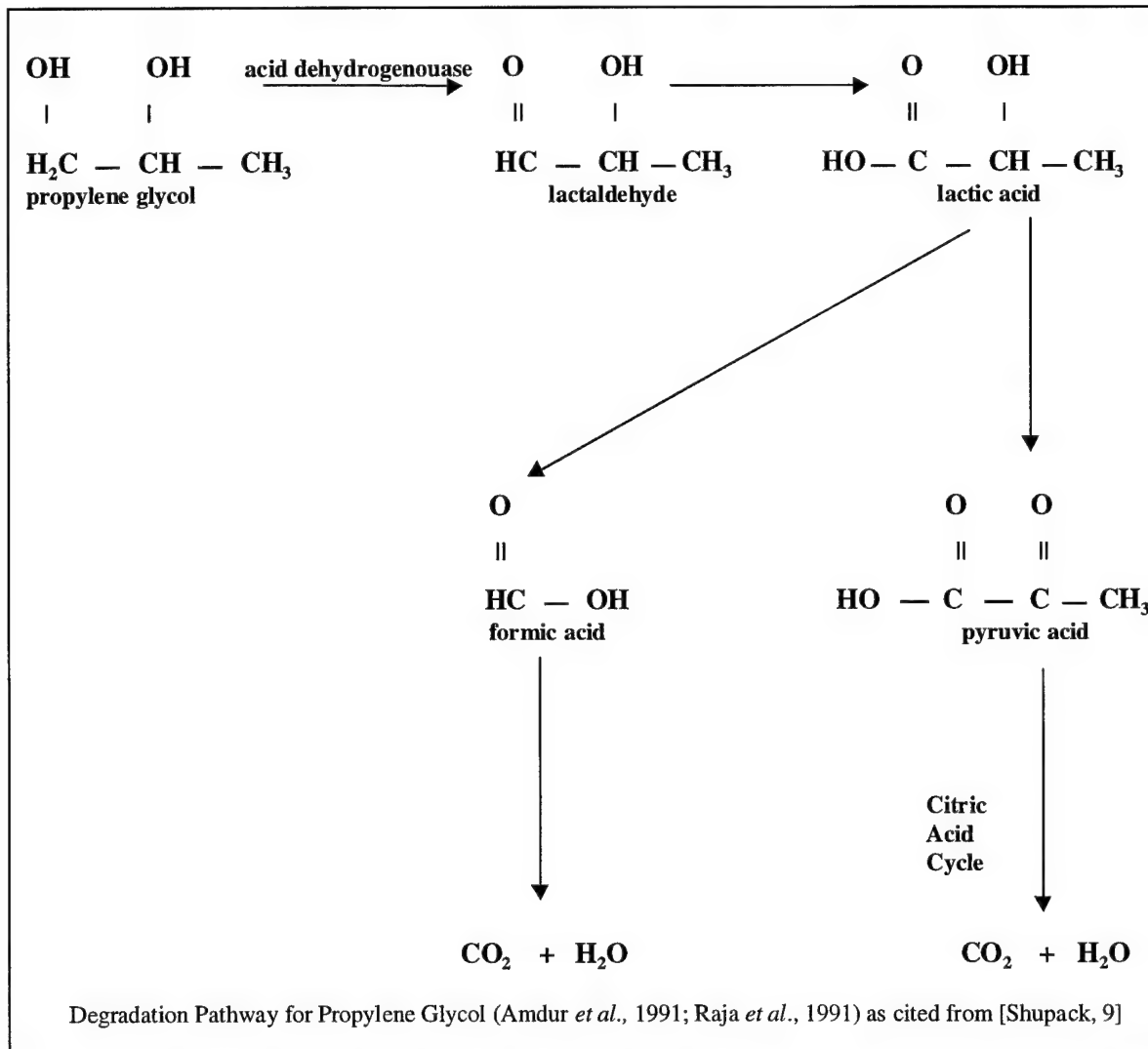
### **2.3.4 Biodegradation of Propylene Glycol**

Propylene glycol is a low-weight-molecular substance, with a simple structure. The simple structure of propylene glycol permits microorganisms in water and soil environments to readily degrade the chemical in both aerobic and anaerobic conditions. Biodegradation has been demonstrated in water [McGahey and Bouwer, 1992], sewage [Jank *et al.*, 1974; Kaplan *et al.*, 1982; Dwyer and Tiedje, 1983; Raja *et al.*, 1991; Nischke *et al.*, 1996], and soils [Haines and Alexander, 1975; Cox, 1978; Klecka *et al.*, 1993, Kawai *et al.*, 1978; Strong-Gunderson *et al.*, 1995; Buasmith and Neufeil, 1996].

Raja *et al.* (1991) used isolated strains of the bacteria *Pseudomonas* and *Aerobacter* to determine possible pathways of degradation. The *Pseudomonas* degraded the propylene glycol too carboxylic and hydroxycarbonic acids. Further decarboxylation to CO<sub>2</sub> was accomplished by the *Aerobacter* strains [Shupack, 7] as shown in Figure 2-1.



Figure 2-1  
Proposed Biodegradation Pathway of Propylene Glycol

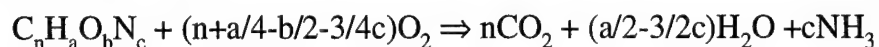


### 2.3.5 Theoretical Oxygen Demand of Propylene Glycol

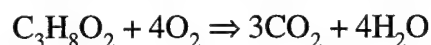
The theoretical oxygen demand (ThOD) for propylene glycol biodegradation may be determined through stoichiometry [Sawyer *et al.*, 528]. The equation in Table 2-3 calculates the amounts (moles) of oxygen to convert an organic carbon material (moles propylene glycol) to carbon dioxide, water, and ammonia.

Table 2-3  
Calculations for the Theoretical Oxygen Demand of Propylene Glycol

Basic Equation for ThOD:



Propylene Glycol ( $C_3H_8O_2$ ) Stoichiometric Equation:



Molar Ratio:  $O_2 : C_3H_8O_2 = 4.0$

Molar Ratio:  $O_2 : CO_2 = 1.333$

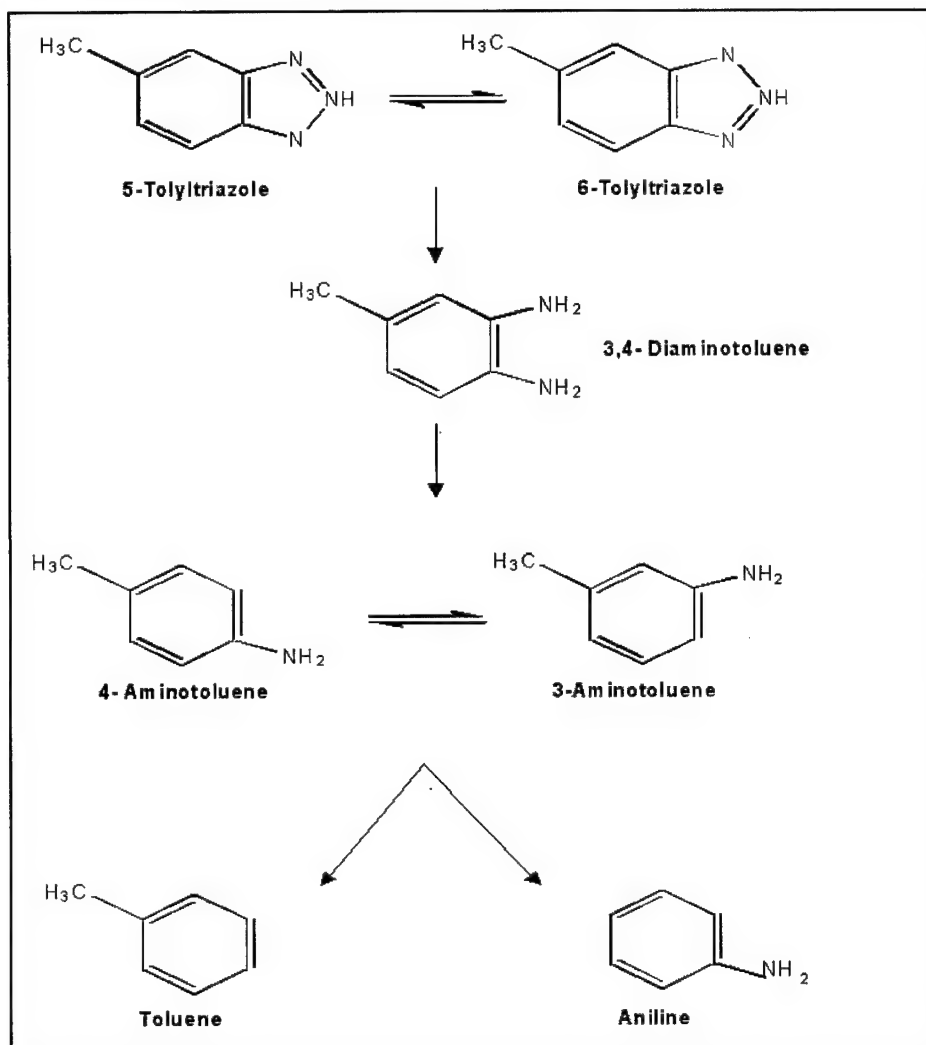
Molecular weight  $C_3H_8O_2 = 76.094$  mg PG/mole

$$\begin{aligned} &\therefore \frac{128 \text{ mg } O_2}{76.094 \text{ mg PG}} \\ &= 1.68 \text{ mg } O_2/\text{mg PG} \end{aligned}$$

### 2.3.6 Biodegradation of Tolyltriazole

The pathway for tolyltriazole biodegradation is still under investigation. It is hypothesized that tolyltriazole degrades anaerobically rather than aerobically [Cornell *et al.*, 1997]. Cornell *et al.* (1997) performed a literature review [Alan R. Katritzky Research Group, 1997; Razo-Flores *et al.*, 1997; Schwarzenbach *et al.*, 1993; Weber, 1994] and proposed the biodegradation pathway shown in Figure 2-2.

Figure 2-2  
Proposed Biodegradation Pathway of Tolyltriazole

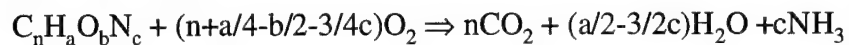


### 2.3.7 Theoretical Oxygen Demand of Tolyltriazole

The theoretical oxygen demand (ThOD) for tolyltriazole biodegradation may be determined through stoichiometry [Sawyer *et al.*, 528]. The equation in Table 2-4 calculates the amounts (moles) of oxygen to convert an organic carbon material (moles tolyltriazole) to carbon dioxide, water, and ammonia.

Table 2-4  
Calculations for the Theoretical Oxygen Demand of Tolyltriazole

Basic Equation for ThOD:



Tolyltriazole ( $C_7H_7N_3$ ) Stoichiometric Equation:



Molar Ratio:  $O_2 : C_7H_7N_3 = 6.5$

Molar Ratio:  $O_2 : CO_2 = .9285$

Molecular weight  $C_7H_7N_3 = 133$  mg TTA/mole

$$\therefore \frac{208 \text{ mg } O_2}{133 \text{ mg TTA}}$$

$$= 1.564 \text{ mg } O_2/\text{mg TTA}$$

### III. Methodology

#### 3.1 Overview of Methods Used

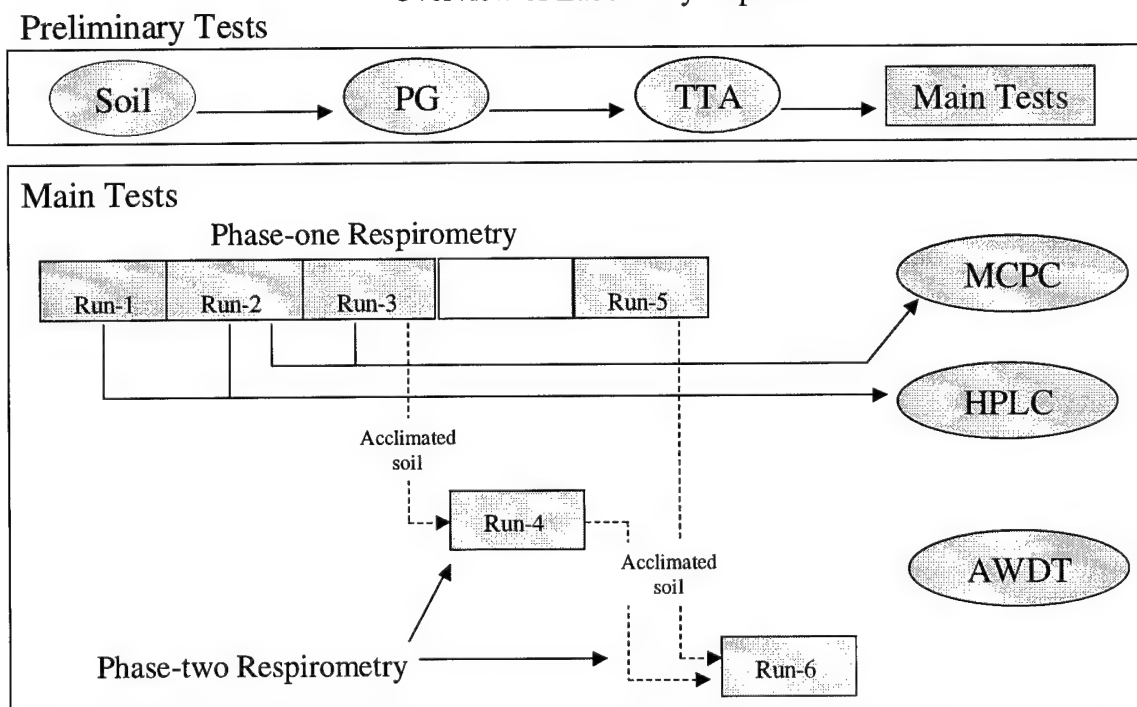
This methodology section describes the materials and procedures used in determining the influence of tolyltriazole on microbial degradation of propylene glycol within a (high-clay) soil. The experiment used in-situ soil microbes to degrade the two ADF chemicals. Microbes activity was monitored by respirometry, which measured oxygen consumption and carbon dioxide production. The specific type of respirometer employed was a Micro-Oxymax<sup>®</sup> respirometer, built by Columbus Instruments, Inc., Columbus, Ohio. Soils were tested at various concentrations and combinations of tolyltriazole and propylene glycol to understand how these ADF components affect microbial degradation.

Once respirometry tests were complete, two additional analyses were performed on selected spent soils. These analysis were HPLC and toxicity tests. HPLC was used to measure residual tolyltriazole on the spent respirometry soil of phase-one. The first toxicity test was a MCPC, which used spent respirometry soil of phase-one. A water-extract of test soil was added to nutrient agar media. The individual cells grew to colonies and allowed a visual count. The colony totals revealed the population of microbes in soil after interaction with the different ADF chemical concentrations. This allowed a correlation of toxicity effects from the ADF chemicals with the respiration data.

The second toxicity test was an AWDT. This test was a stand-alone test of varying ADF chemical concentrations and combinations. Nutrient agar plates were

allowed to solidify and a microbe rich solution (uncontaminated soil based) was spread on the surface of the nutrient agar. A small well was placed in the center of the agar material and filled with a particular test chemical (propylene glycol, tolyltriazole, or both). The microbes were incubated and colony formations were observed. Suppression of colony formation near the agar well suggested toxicity. An overall layout of all laboratory methods is shown in Figure 3-1. The different chemical treatments for each respirometry run are listed in Appendix E, Table E-1.

Figure 3-1  
Overview of Laboratory Experiments



## 3.2 Laboratory Procedures

### 3.2.1 Soil Selection

ADF component degradation was analyzed in both a sandy soil and a high-clay soil by Johnson (1997) and O'Malley (1997). Their results showed appreciably more

degradation of propylene glycol in high clay soil rather than sandy soil. This investigation used the same high-clay soil.

### **3.2.2 Soil Collection**

The natural soil in the Dayton, Ohio area is clay based. An open grassy area was selected adjacent to the wooded area that Johnson (1997) and O'Malley (1997) used in their research. A new location was selected in hope that increased microbial population and variety would be found in the grassy area. In many studies, the quantities of microorganisms are significantly less in wooded areas when compared to open grassy areas [Whitman *et al.*, 6578]. In addition, the experiments were designed to model airfield conditions whenever possible.

Soil was collected on September 5, 1998 with sunny-temperate conditions of 31°C and high humidity. The collection was performed with a steel shovel and an 8 liter (2-½ gallon) plastic bucket. Both were pre-cleaned with de-ionized water prior to soil collection. The majority of grass and humic matter was stripped from the collection area within the first 6 cm. The usable soil was collected within the next 20 – 30 cm (vertical layer), in an area of approximately 0.5 square meters. There was no unusual odor or debris encountered during collection. The soil sample was placed in the bucket and covered. The lid was not sealed in order to maintain an aerobic condition. No further soil collections were required, since the 8 liters provided an adequate amount of soil for all of the experimental research.

### 3.2.3 Soil Preparation

The method described by Klecka *et al.* (1993) was followed. Their method required the soil to be pre-cleaned of large organic matter and sieved through a No. 8. U.S.A. standard testing sieve. A 2 mm square wire mesh was used in place of a No 8. sieve for removal of foreign matter such as leaves, stones, roots, and visible insects.

Experimental runs were conducted over a six-month period. The soil was carefully stored to maintain the quality of soil and microorganisms over this period. The prepared soil was immediately placed in plastic bags (Ziplock™) and refrigerated at  $4 \pm 1^{\circ}\text{C}$  to slow microbial activity and minimize changes.

### 3.2.4 Soil Characteristics

The Soil, Water, and Plant Testing Laboratory, Colorado State University, Ft Collins, Colorado, performed an independent analysis of the soil used in the investigation. As indicated in the report (Appendix A), all of the essential nutrients were in ample amounts for support of microbial metabolism. The results from the laboratory are summarized in Table 3-1.

Table 3-1  
Chemical Characteristics of the Soil

| Organic Matter<br>(%) | Phosphorus (P)<br>(ppm) | Potassium (K)<br>(ppm) | (Mg)<br>(ppm) | Calcium (Ca)<br>(ppm) | pH  |
|-----------------------|-------------------------|------------------------|---------------|-----------------------|-----|
| 2.9                   | 5.3                     | 94.3                   | 2.8           | 3.0                   | 7.8 |

The physical characteristics were also analyzed. The results from the independent soil report are summarized in Table 3-2.



Table 3-2  
Physical Characteristics of the Soil

| % Sand | % Silt | % Clay | ASTM Soil Classification |
|--------|--------|--------|--------------------------|
| 48     | 36     | 16     | Loam                     |

### 3.2.5 Soil Moisture

As discussed earlier, microbial metabolism is directly related to the water content in the soil. Water content tests used by Thomas (1996) were followed to determine the percentage of field capacity (saturated soil moisture). Preliminary tests were performed to determine the optimal water content that would provide adequate mixing/workability of this soil. Soil above 65% field capacity showed clumping and compaction. This was considered unacceptable (potentially anaerobic conditions). The range of 55 – 65% field capacity was established as usable. The final choice of a 60% field capacity was set, and water/solution was added to achieve this level within all the experimental runs.

The reason for beginning all experiments at a relatively high water content arises from the operation of the respirometer. Once the microcosms were closed, no further injections of fluids occurred during an experimental run. Evaporation of water occurred as the respirometer passed dried air over the soil during headspace sampling. Soil moisture tests were performed on the spent soil after respirometry runs. The data revealed an average range of 50 – 55% field capacity after respirometry runs.

### 3.2.6 Soil pH

The untreated soil had revealed a pH of 7.8 for the soil as reported in the independent soil analysis. No adjustment of pH was done prior to respirometry

experiments due to its near neutral condition. Simple pH tests were conducted before and after the respirometry tests. The data was summarized in Table 3-3.

Table 3-3  
Tests on Soil pH used in Respirometry Runs

| Soil Treatment                           | Respirometry Test |       | Instrument                    |
|--|-------------------|-------|-------------------------------|
|  | Before            | After |                               |
| De-ionized H <sub>2</sub> O              | 7.8               | 7.8   | HACH<br>pH tester<br>44450-00 |
| PG <sub>1000</sub>                       | 7.9               | 7.8   |                               |
| PG <sub>1000</sub> & TTA <sub>1000</sub> | 7.9               | 7.8   |                               |

### 3.2 Treatment Overview

Respirometry experiments were conducted in two phases. Phase-one used uncontaminated soil with varied combinations of ADF chemicals and concentrations. Phase-two used acclimated soil/microorganisms from phase-one tests.

#### 3.2.1 Overview of Treatment Layout for the Respirometer

There are 20 microcosms available within the Micro-Oxymax<sup>®</sup> respirometer. Phase-one used five microcosms for each treatment type (PG alone, TTA alone, PG & TTA) in experimental runs, along with three microcosms for blank treatments (de-ionized H<sub>2</sub>O). Two empty bottles were also used to monitor machine noise and variation. Phase-two used a range of three to five microcosms due to the various treatments and data requirements. Appendix E, Table E-1 contains a detailed layout of all respirometry runs and treatments.

Sampling of high respiration microcosms (propylene glycol in soil) just before sampling low respiration microcosms (blank soil) can be problematic due to carry-over.

The high CO<sub>2</sub> and low O<sub>2</sub> in the sampling ports/sensors/tubing from the first measurement can reduce affect the next microcosm measurement. In an attempt to minimize the effect, an optimal sampling configuration was developed. An example of an optimal bottle layout in shown in Table 3-4.

Table 3-4  
Example of Respirometry Treatment Layout: Phase-one, Run-1

|           |  |  |  |  |  |
|-----------|--|--|--|--|--|
| Bottle    | 1                                      | 2                                      | 3                                      | 4                                      | 5                                      |
| Treatment | TTA <sub>25</sub>                      | TTA <sub>25</sub>                      | TTA <sub>25</sub>                      | TTA <sub>25</sub>                      | TTA <sub>25</sub>                      |
| Bottle    | 6                                      | 7                                      | 8                                      | 9                                      | 10                                     |
| Treatment | Empty                                  | Empty                                  | PG <sub>1000</sub> & TTA <sub>25</sub> | PG <sub>1000</sub> & TTA <sub>25</sub> | PG <sub>1000</sub> & TTA <sub>25</sub> |
| Bottle    | 11                                     | 12                                     | 13                                     | 14                                     | 15                                     |
| Treatment | PG <sub>1000</sub> & TTA <sub>25</sub> | PG <sub>1000</sub> & TTA <sub>25</sub> | Soil                                   | Soil                                   | Soil                                   |
| Bottle    | 16                                     | 17                                     | 18                                     | 19                                     | 20                                     |
| Treatment | PG <sub>1000</sub>                     | PG <sub>1000</sub>                     | PG <sub>1000</sub>                     | PG <sub>1000</sub>                     | PG <sub>1000</sub>                     |

As the layout demonstrates, treatments of 1,000 mg/kg propylene glycol alone (PG<sub>1000</sub>), 25 mg/kg tolyltriazole alone (TTA<sub>25</sub>), and a combination of propylene glycol and tolyltriazole (PG<sub>1000</sub> & TTA<sub>25</sub>) are separated by either empty or blank soil microcosms.

### 3.3.2 Phase-one Treatments

Phase-one used ADF chemicals on uncontaminated (high-clay) soil. The phase-one tests are associated with experimental Run-1, Run-2, Run-3, and Run-5. The choices in ADF chemical concentrations and combinations were developed through preliminary research. Section 3.3.4 provides further explanation on the preliminary research of concentration choices.

### **3.3.3 Phase-two Treatments**

Phase-two respirometry experiments measured the response of acclimated microorganisms from phase-one soil. Propylene glycol at 1,000 mg/kg was the only ADF chemical and concentration that was reapplied. Set-up and choice of phase-two treatments were developed from results of phase-one respirometry data. The phase-two tests are associated with experimental Run-4 and Run-6.

### **3.3.4 Microcosm Preparation for Respirometer Analysis**

As stated earlier, the workable field capacity was established at ~60% from preliminary tests. Previous experiments by Johnson (1997) and O'Malley (1997) have shown that during periods of rapid respiration the O<sub>2</sub> levels fell below the respirometers lower-detection limit (19.29% O<sub>2</sub>). The O<sub>2</sub> depletion was due to large soil amounts (thus many microbes) and high concentrations of propylene glycol (food source).

Shortening the sampling interval and lengthening the duration of refreshing O<sub>2</sub> was considered. However, the respirometer cycle time was already near six hours for the 20 microcosms. Microbial respiration rate was the only other parameter to adjust.

The preliminary tests showed a soil mass of 50 gm along with a propylene glycol concentration of 1,000 mg PG/1 kg soil would be optimal. The 50 grams at 60% field capacity soil would consist of 45 grams of uncontaminated soil (semi-dry) with 5 mL (5 gm) of solution. Calculations are provided in Appendix B.

Tolyltriazole solubility in water and water-propylene glycol solutions were tested to determine their interaction. The interaction being tested was the ability for tolyltriazole to dissolve equally in both base liquids. A consistent solution (no

granules/flocculent) of tolyltriazole was desired in the solution for accuracy in the treatment dose of soil. The interactions were measured through range finding tests of concentrations and temperatures, summarized in Table 3-5.

Table 3-5  
Tolyltriazole Saturation Points in Aqueous Solution

| Concentration of TTA                    | 5,000 mg/L | 5,250 mg/L | 5,500 mg/L | 5,750 mg/L | 6,000 mg/L | 6,250 mg/L  |      |
|---|------------|------------|------------|------------|------------|-------------|------|
| TTA in 10,000 mg/L PG solution          | No Flocc   | Flocc      | Flocc      | Flocc      | Flocc      | Flocc       | 4°C  |
| TTA in de-ionized H <sub>2</sub> O Only | Flocc      | Flocc      | Flocc      | Flocc      | Flocc      | Flocc       |      |
| Concentration of TTA                    | 7,500 mg/L | 8,000 mg/L | 8,500 mg/L | 9,000 mg/L | 9,500 mg/L | 10,000 mg/L |      |
| TTA in 10,000 mg/L PG solution          | No Flocc   | No Flocc   | No Flocc   | No Flocc   | No Flocc   | No Flocc    | 43°C |
| TTA in de-ionized H <sub>2</sub> O Only | No Flocc   | No Flocc   | Flocc      | Flocc      | Flocc      | Flocc       |      |

Note: After 43°C heat is removed, TTA precipitates out of both solutions



 = initial flocculent (floc) of TTA granules in solution  
 = heavy flocculent (floc) of TTA granules in solution

Table 3-5 reveals that tolyltriazole did not flocculate in water or water-propylene glycol up to the 5,000 mg/L at 25°C. The application of heat allowed higher concentrations of tolyltriazole to dissolve in the solutions, which allowed consistent solution concentrations for application on soil.

To prevent chemical and microbial degradation of the solutions between experimental runs, a protocol of generating fresh batches of solution was adopted. The calculations of mass and volumes for preparing the concentrations of ADF solutions are found in Appendix C.

The propylene glycol solution (10,000 mg/L) was prepared from a reagent grade (Mallinckrodt OR, 1925; 1,2-Propanediol) chemical to ensure purity and concentration. Five grams of propylene glycol was diluted into 500 mL of de-ionized water in a volumetric flask (Pyrex®), with a ground glass stopper. It was mixed with a magnetic

stirrer (Corning™, PC -210) for approximately one hour, at room temperature (~22°C) in lighted conditions.

The tolyltriazole only solutions (250 – 7,500 mg/L) were prepared from commercial grade COBRATEC TT-100 (sample 4239701). Solid phase pellets of the tolyltriazole were ground into powder in a pre-cleaned crucible. The appropriate amounts of the powder were mixed with 200 mL of de-ionized water in a volumetric flask, then mixed on a heated/electro-magnetic stirrer (PMC™, 525A).

- Concentrations of 250 – 5,000 mg/L were maintained at ~22°C (room temperature) and stirred for eight hours in unlighted conditions.
- Concentrations of 5,000 – 10,000 mg/L were heated to 43°C for 15 minutes, then stirred for eight hours in unlighted conditions at ~22°C, then reheated to 43°C for 15 minutes prior to application on the soil.

The combined solution of propylene glycol with tolyltriazole was then prepared with the same chemicals. The selected amount of tolyltriazole was added to 200 mL of propylene glycol solution (10,000 mg/L) and mixed in a volumetric flask with a ground glass stopper. The chemicals were mixed upon an electro-magnetic stirrer for approximately eight hours, at the appropriate temperature, as related to the tolyltriazole concentration in unlighted conditions.

The soil was allowed to adjust to room temperature (~22°C) in advance of mixing with solutions. The acclimatized soil required less time to equilibrate at the respirometers incubator temperature (25°C).

The respirometers microcosm bottles (250 mL, Pyrex) were pre-cleaned with de-ionized water. The soil and 5 mL of test solution (de-ionized water, propylene glycol, tolyltriazole, or propylene glycol with tolyltriazole) was added to the bottle and stirred.

A stainless steel spatula was used to mix the contents for five minutes per microcosm. This ensured all soil was fully mixed and wetted. The spatula was cleaned with de-ionized water before mixing other microcosm/treatments.

### **3.4 Respirometer**

#### **3.4.1 Overview of Respirometer System**

The respirometer used a “closed loop” system configuration for measuring O<sub>2</sub> consumption and CO<sub>2</sub> production gases from each individual microcosm. Details on use of the Micro-Oxymax<sup>®</sup> respirometer may be found in Totten (1995), Baker (1995), and Thomas (1996).

#### **3.4.2 Respirometer Calibration**

Prior to each experimental run, several calibration adjustments were performed to ensure accurate O<sub>2</sub> and CO<sub>2</sub> measurements. The CO<sub>2</sub> sensor was zeroed through the introduction of 99.999% pure nitrogen (PRAXAIR Company, certified mixture). The nitrogen-only atmosphere ensured a zero reference point for calibration. Then a laboratory grade (Liquid Carbonic Company) mixture of CO<sub>2</sub> (0.501%) and O<sub>2</sub> (20.4%) was introduced. The CO<sub>2</sub> and O<sub>2</sub> sensors were adjusted to match the standard gas and then set/locked for the remainder of the experimental run. Each new experimental run required re-calibration prior to initiating the respirometers automated sampling program.

Leak checks of each microcosm (250 mL bottle and tubing) were performed by the machine through a self-diagnostic program that verifies “pass or fail” of all the systems. The “passing” range of  $\pm 0.2$  mL/min leakage is allowed for one out of three

times tested on each of the 250 mL bottles tested [Micro-Oxymax<sup>®</sup> Software manual, 19].

In response to a recommendation from Johnson (1997) and O'Malley (1997), the respirometer was relocated to a climate-controlled laboratory. The purpose was to reduce atmospheric humidity and temperature variations that seemed to cause erratic calibration checkouts. In addition, the oxygen sensor was replaced on July 19, 1998, since O<sub>2</sub> sensitivity began to rise above specified limits. The machine was then inspected and calibrated at Columbus Instruments on August 21, 1998 to ensure the machine met factory tolerances.

### **3.4.3 Respirometer Parameter Controls**

The experimental runs were conducted during a two-week period using controlled environmental parameters. Temperature was maintained in an incubator (Lab Line<sup>™</sup>, AMBI-HI-LO) at  $25 \pm 1^{\circ}\text{C}$ . Photo-degradation was eliminated throughout all experimental runs, since the incubator shielded the microcosms from light. The refreshed air provided to the respirometer was passed through a two-stage moisture absorbent system. First, through a stand-alone absorbent system (DRIERITE<sup>™</sup>, CaSO<sub>4</sub>) then through a desiccant, containing magnesium perchlorate (GFS chemical, Mg(ClO<sub>4</sub>)<sub>2</sub>). Low moisture air was required for accurate measurements of CO<sub>2</sub>.

### **3.4.4 Data Collection and Conversion**

The experimental software (Micro-Oxymax<sup>®</sup> V6.03) for the respirometer provided detailed information/data for automated sampling. Every six hours a sample point was captured for each of the 20 microcosm bottles for the entire two-week range of



the experimental run. Table 3-6 summarizes the respirometers measurements.

Table 3-6  
Respirometer Output Data

| Title     | Used for Data Analysis     |                            |                                    |                                    | Used to ensure machine is functioning properly and within ranges desired |                  |                   |
|-----------|----------------------------|----------------------------|------------------------------------|------------------------------------|--|------------------|-------------------|
|           | O <sub>2</sub> Consumption | CO <sub>2</sub> Production | Rate of O <sub>2</sub> Consumption | Rate of CO <sub>2</sub> Production | Temperature  | % O <sub>2</sub> | % CO <sub>2</sub> |
| Units     | (uL)                       | (uL)                       | (uL/hr)                            | (uL/hr)                            | °C   |                  |                   |
| Precision | 0.1                        | 0.1                        | 0.1                                | 0.1                                | 0.01   | OK if >19.29%    | All Ranges        |

**Note:** If O<sub>2</sub>% falls below 19.29%, the machine cannot account for the actual O<sub>2</sub> volume for the sample interval.

### 3.5 High Performance Liquid Chromatography (HPLC)

#### 3.5.1 Overview of HPLC Detection of Tolyltriazole

HPLC analysis was performed on a Hewlett Packard 2170 HPLC using ultraviolet detection. The HPLC used a Hewlett Packard auto-sampler in conjunction with software support of Hewlett Packard Chem-Station<sup>®</sup> for liquid chromatography systems (Rev. A. 4.02). The HPLC analysis was used to measure the residual tolyltriazole absorbed on the soil after respirometry. HPLC tests were also performed on freshly inoculated soil, which was immediately processed/extracted in an attempt to measure dissolved phase of tolyltriazole in the soil. The analysis of residual amounts of tolyltriazole before and after respirometry tests aided in identifying as many degradation pathways (physical, chemical, and biotic) as possible.

#### 3.5.2 Extraction Method for Tolyltriazole in Soil

Approximately 12 – 13 gm of soil was placed in a 40 mL bottle (Fisher Brand, EPA vials). 15 mL of methanol (Fisher Chemicals, HPLC Grade) was then added to the

soil for extracting the remaining tolyltriazole. The 40 mL bottles were mixed on a rotator (Glas-Col, Laboratory Rotator) for 24 hours and then centrifuged (International Clinical, Model 4182C) for 20 minutes at a speed of 1,000 rpm. Upon completion, the liquid phase of the sample was carefully extracted and filtered (Gelman Sciences Acrodisc, 0.2  $\mu$ m filter). The sample was then ready for HPLC analysis.

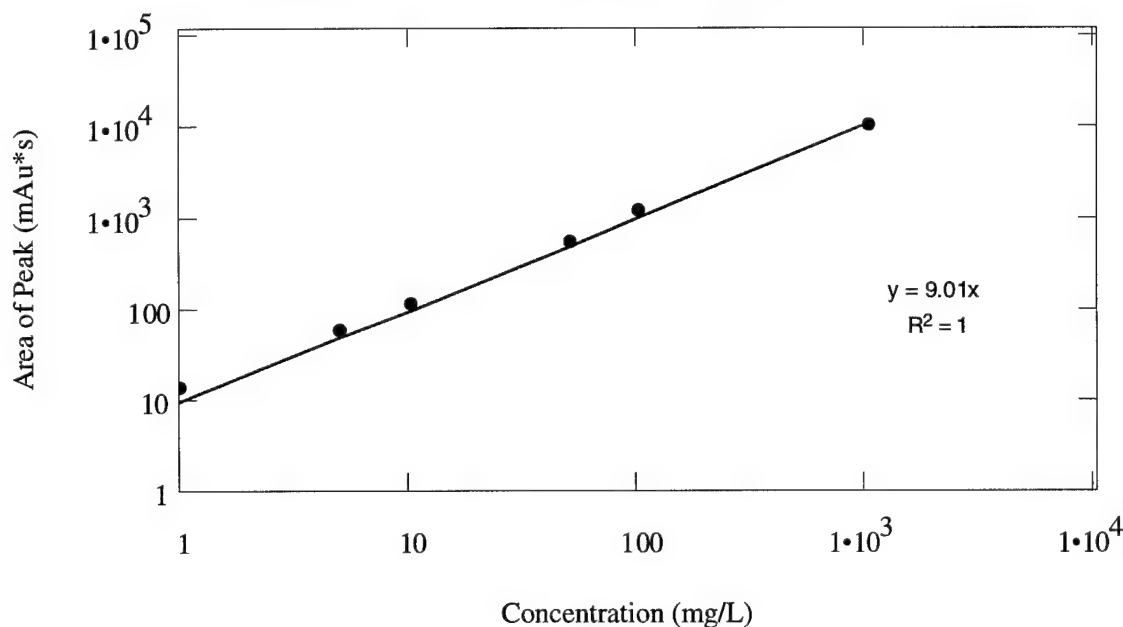
### **3.5.3 HPLC Detection Method for Tolyltriazole**

After filtration of the samples, they were injected into a valve fitted 100  $\mu$ m loop. The injection volumes were 10  $\mu$ L, and the tolyltriazole was detected at wavelength of  $280 \pm 2$  nm. The separation was carried out at room temperature ( $\sim 22^{\circ}\text{C}$ ) with the diode array temperature set at room temperature ( $\sim 22^{\circ}\text{C}$ ). The column used was an Altech<sup>®</sup> Adsorbanosphere C8 5U (250 mm x 4.6 mm). The mobile phase used two different solvents; a phosphate buffer composed of 0.5 mL phosphoric acid ( $\text{H}_3\text{PO}_4$ ) and 0.65 gm potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) in one liter of de-ionized water, along with HPLC grade methanol. The solvents were set-up in a ratio and gradient that allowed for the tolyltriazole to peak at a reasonable time (8 min) and then flushed the column of any residual organics. The solvent ratio started at 30:70 (buffer:methanol) and transitioned to 50:50 (buffer:methanol) in the first 10 minutes, via the automated controls. At the 10 minute point, the ratio increased immediately to 10:90 (buffer:methanol) and stayed constant for the next 15 minutes in order to flush the organics from the system. The above method was used by Johnson (1997) and developed by PMC Specialties Group, Inc, of Cincinnati, Ohio.

### 3.5.4 Calibration Curve for HPLC Detection of Tolyltriazole

The concentrations used for establishment of the calibration curve were varied from 1 mg/L to 1,000 mg/L. The concentrations were prepared using the same tolyltriazole material with a base solution of methanol. The HPLC detection areas, identified as microabsorbency units \* second (mAu\*s), were calculated for each concentration (mg/L) with the HP Chem-station software. The calibration curve was then fitted with a linear regression line that possessed a  $R^2 = 1.00$  (Pearson coefficient). Figure 3-2 depicts the calibration curve plotted in log/log scale for convenient interpretation and conversion of the HPLC detection areas (mAu\*s) to concentrations (mg/L).

Figure 3-2  
Calibration Curve for Tolyltriazole Detection with HPLC



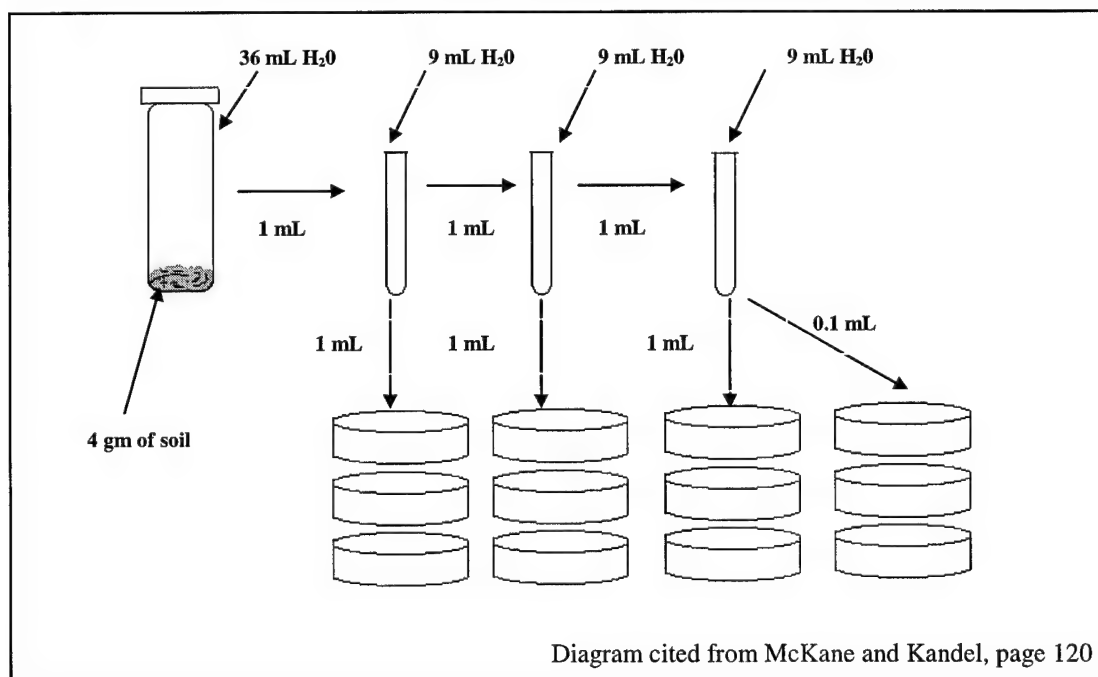
**Note:** The limit of detection (LOD) was determined at  $\pm 3$ mg/L. Appendix D lists all data and calculations for the calibration curve and LOD.

### 3.6 Microbial Colony Plate Count (MCPC)

#### 3.6.1 Overview of MCPC Test

The method of microbial colony plate counting used a simple measurement of the number of living microbes and their health in soil. Theoretically, each healthy cell forms a single colony on the solid medium that can support its growth. After incubation, the number of colonies on the plate ideally equals the number of cells in the sample inoculated on the agar [McKane and Kandel, 121]. The plate counts must be sufficiently diluted prior to injection on the nutrient agar plate. The diluted sample provides sufficient area for colonies to grow separately. This allowed definitive counts of the individual populations. An overview of the test set-up is shown in Figure 3-3.

Figure 3-3  
Overview of Microbial Colony Plate Count Test



Three replicate MCPC tests (petri dishes) were preformed for each dilution. The MCPC method was applied to soils exposed to various concentrations of ADF

components. This helped determine the influence of the chemicals on the health and activity of the microbe populations.

### **3.6.2 Set-up of Materials for the MCPC Test**

The preparation of nutrient agar plates followed *Standard Methods* protocols. The nutrient agar (Difco, Bacto™) was pre-sterilized at 121°C for 15 minutes in an autoclave. The dilution water was prepared with sodium chloride (NaCl) at 0.5 gm for one liter de-ionized water. This prevented the rupture of microbial cell membranes due to the osmotic pressure difference. The petri dishes (Fisher Brand, 95 x 15 mm) were pre-sterilized disposable-plastic. Incubation of the inoculated plates occurred for 2 – 3 days at 25°C in an incubator oven.

### **3.6.3 Counting Techniques for MCPC**

Plates were examined at 12 hour intervals within the 2 – 3 day time period. The actual counting was done subjectively on a lighted colony counter (Leica™, Model 3327). The optimal time for the visual identification of microbial populations was at the 48 hr point. After 48 hours, the size and abundance of growths upon plates reduced the accuracy of counting. The ranges of normally accepted population counts on a plate is typically established between 30 – 300 individual colonies [Eaton *et al.*, 9-33].

## **3.7 Agar Well Diffusion Test (AWDT)**

### **3.7.1 Overview of AWDT**

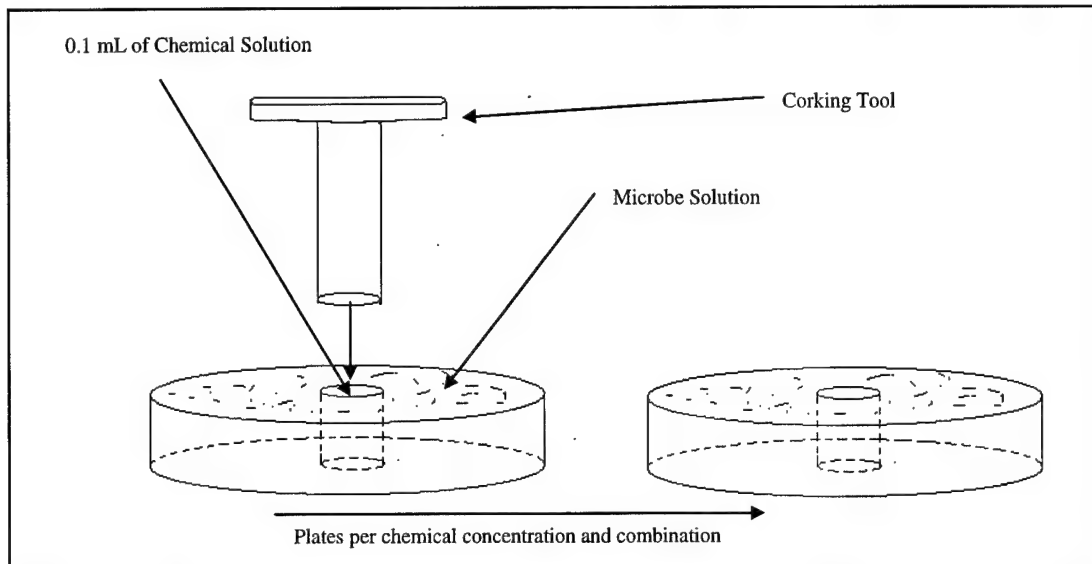
The agar well diffusion test is used to measure whether a chemical supports or

inhibits microbial growth and activity. The nutrient agar was used as a reliable food source to ensure a healthy population of microorganisms. A holding well was dug out of the agar media in the center of the prepared petri dish. Individual and combined ADF chemical solutions were prepared and placed in the well to allow diffusion onto the agar and newly introduced microorganisms. The microbes were allowed to incubate and interact with the chemicals. The inhibition or proliferation of microbial colonies around the well was used to measure toxicity. If microbes exist in and around the well area, then the chemical concentration is apparently not toxic to them. If microbial colonies formed a measurable distance away from the well area, then toxicity is apparent. A toxicity test similar to this is described in the *Handbook of Environmental Microbiology* [Mills, 355].

### **3.7.2 Set-up of Materials for the AWDT**

Nutrient agar is prepared within an autoclave at 121°C for 15 minutes as described above in section 3.6.2. The agar was then poured into pre-sterilized petri dishes and allowed to solidify for one hour. Using a “corking tool” (pre-sterilized) a small well was placed in the center of the agar. A microbial rich solution is prepared and spread upon the plate surface. Individual and combined ADF chemical solutions of propylene glycol (10,000 mg/L), tolyltriazole (5,000 – 10,000 mg/L), or propylene glycol (10,000 mg/L) with tolyltriazole (5,000 – 10,000 mg/L) were prepared and used to fill (~0.1 mL) the well. The layout of ADF chemical concentrations and combinations is located in Appendix J. The petri dishes incubated at 25°C for several days and were monitored for signs of toxicity around the well on a 12 hour basis. The AWDT used several plates per chemical treatment. See Figure 3-4 for an overview of the layout.

Figure 3-4  
Overview of Agar Well Diffusion Test



### 3.8 Statistical Methodology

The first research objective was to determine the impact on microbial biodegradation of individual ADF chemicals on an uncontaminated soil environment. This determination was made using the  $O_2$  consumption totals of the contaminated soil (PG alone or TTA alone) against the uncontaminated soil (blank soil). A two-sample t-test was used to measure the difference of  $O_2$  total means (chemical treatment on soil minus the blank) using a significance level of  $\alpha = 0.05$ . The null hypothesis was that there was no effect on  $O_2$  consumption due to contaminants addition. The t-test results were converted into a 95% confidence interval (CI) for the entire respirometry run period (336 hrs). The CI was graphed to provide a visual explanation of increased  $O_2$  consumption (biodegradation) or decreases (inhibition). Appendix F contains a detailed layout of the statistical set-up, formulas, and figures.

The second research objective was to determine the impact on microbial

biodegradation due to the combined ADF chemical treatment (PG & TTA) on an uncontaminated soil environment. The null hypothesis states that there was no difference in O<sub>2</sub> consumption due to combined ADF components compared against the individual ADF components on uncontaminated soil. This determination was made using the mean O<sub>2</sub> consumption totals of the contaminated soil (PG & TTA) against a linear combination of individual treatments (PG alone, TTA alone, and blank) on uncontaminated soil. A two-sample t-test was used to measure the difference of mean O<sub>2</sub> totals using a significance level of  $\alpha = 0.05$ . The t-test results were converted into a 95% CI for the entire respirometry run period (336 hrs). The CI provided a visual depiction for the amount of O<sub>2</sub> consumption increases or decrease due to the combined ADF components. See Appendix G for a detailed layout of the statistical set-up, formulas, and figures.

The third research objective was to compare ADF pre-treatment/pre-conditioning of the same soil for biodegradation activity. This objective was checked with the initial O<sub>2</sub> consumption rates (using ThOD calculations to develop the initial biodegradation rates) from propylene glycol (1,000 mg/kg) application on uncontaminated soil (unconditioned microbes) against pre-contaminated soil (microbes acclimated to propylene glycol). The statistical test method used a two-sample t-test with a significant level of  $\alpha = 0.05$ . The null hypothesis was that there was no difference between the initial biodegradation rates of acclimated soil compared to uncontaminated soil once propylene glycol (1,000 mg/kg) was applied. See Appendix L for a detailed layout of the statistical test method.



## **IV. Data Analysis**

### **4.1 Overview of Data Analysis**

Two forms of analyses were performed on the data; visual and statistical. Visual and statistical analyses were conducted on both phase-one and phase-two respirometry data. Statistical tests were done on HPLC results and visual analysis was conducted on both the MCPC and the AWDT data.

### **4.2 Repeatability/Consistency of Laboratory/Respirometry Procedures**

A comparison/review of all six experimental runs was performed prior to analyzing the respirometry data for biodegradation effects. The goal was to show consistency and repeatability of the respirometer/laboratory procedures used throughout experimental runs that comprised the research. Once accuracy/quality was assured in the respirometer measurements and proper laboratory techniques, the focus moved to analyzing the data for microbial affects from the ADF components.

The checks for respirometry measurements and laboratory procedures used a comparison of similar treatments within the respirometry runs. The statistical tests were performed with a one-way analysis of variance (ANOVA) using a P-value and F-test. The one-way ANOVA results were then used to generate a Tukey-pairwise test of the mean O<sub>2</sub> consumption totals for each respirometry run. This was used to identify any possible irregularities in respirometry runs.

There were two specific soil treatments replicated in the experiments. First, de-ionized H<sub>2</sub>O (blank) was used in three runs. Then 1,000 mg/kg of propylene glycol

(PG<sub>1000</sub>) was used in five runs. The repeatability and performance of the respirometer were performed through comparison of blank treatments on uncontaminated soil. Consistency in laboratory procedures and techniques was determined through the PG<sub>1000</sub> treatments used in respirometry runs. If preparation of solutions were incorrectly performed, then a significant difference in O<sub>2</sub> consumption would develop, thus eliminating the respirometry run from analysis.

The cumulative O<sub>2</sub> consumption totals (μL) at the 288 hr point, for both blank and PG<sub>1000</sub> treatments, were obtained from all respirometry runs. The statistical tests for each soil treatment were generated with STATISTIX<sup>®</sup> 4.0 software using a significance level of  $\alpha = 0.05$ . The null hypothesis stated that for the replicated test conditions, there was no difference in respirometry runs (mean O<sub>2</sub> consumption totals, 288 hr point).

#### **4.2.1 Statistical Test of Blank Respirometry Runs for Repeatability/Consistency**

There were three microcosm bottles in each of the three runs to compare. The O<sub>2</sub> consumption totals for each respirometry run were compared for outliers, using a Box and Whiskers plot. The plot showed no outliers. The residuals for each respirometry run were calculated and plotted on a Wilk-Shapiro/Rankit plot of residuals. The data appeared to have aptness from the Wilk-Shapiro statistic = .853 (acceptable).

An F-test value and P-value were determined from the one-way ANOVA. The results of the tests are summarized in Table 4-1.

Table 4-1  
One-way ANOVA results for De-ionized H<sub>2</sub>O on Uncontaminated Soil (288 hr point)

| Test                         | Testing Values<br>(Devore, 709) | Test Results<br>STATISTIX 4.1 | Results                |
|------------------------------|---------------------------------|-------------------------------|------------------------|
| $f^* > F_{crit}$ Reject Null | $F_{crit} = 5.14$               | $f^* = 0.69$                  | Do not reject the Null |
| $P < \alpha$ Reject Null     | $\alpha = 0.05$                 | $P = 0.5339$                  | Do not reject the Null |

See Appendix M, page M-3 for results

The null hypothesis was not rejected, thus stating the blank (de-ionized H<sub>2</sub>O) soil treatments have shown that the respirometer maintained repeatable/consistent measurements. Table 4-2 contains the Tukey-pairwise comparison of means from the one-way ANOVA results.

Table 4-2  
Consistency of Respirometry Runs using a Tukey-pairwise Comparison of O<sub>2</sub> Mean Totals (Blank on Uncontaminated Soil, 288 hr point)

| RUN   | MEAN | HOMOGENEOUS<br>GROUPS |
|---|------|-----------------------|
| 1   | 8264 | I                     |
| 2   | 8048 | I                     |
| 3   | 7881 | I                     |
| THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES<br>AMONG THE MEANS. |      |                       |

The comparison (Table-4-2) shows consistency in all the O<sub>2</sub> mean totals tested and confirms the F-test and P-value acceptance of the null hypothesis (Table 4-1).

#### 4.2.2 Statistical Test of PG<sub>1000</sub> Respirometry Runs for Repeatability/Consistency

Three to five microcosm bottles were compared in each of the five runs. The O<sub>2</sub> consumption totals at the 288 hr point for respirometry runs were compared for outliers, using a Box and Whiskers plot. The plot showed no outliers. The residuals for each

respirometry run were calculated and plotted on a Wilk-Shapiro/Rankit plot of residual. The data appeared to have aptness from the Wilk-Shapiro statistic = .995 (acceptable).

An F-test value and P-value were obtained from the one-way ANOVA. The degrees of freedom were calculated and the F-critical ( $F_{crit}$ ) value was determined. The results of the tests are summarized in Table 4-3.

Table 4-3  
One-way ANOVA results for PG<sub>1000</sub> on Uncontaminated Soil (288 hr point)

| Test                         | Testing Values<br>(Devore, 709) | Test Results<br>STATISTIX 4.1 | Results         |
|------------------------------|---------------------------------|-------------------------------|-----------------|
| $f^* > F_{crit}$ Reject Null | $F_{crit} = 2.87$               | $f^* = 54.87$                 | Reject the Null |
| $P < \alpha$ Reject Null     | $\alpha = 0.05$                 | $P = 0.000$                   | Reject the Null |

See Appendix M, page M-5 for results

The null hypothesis was rejected, thus stating the propylene glycol soil treatment/runs have shown inconsistency. This prompted the completion of a Tukey-pairwise comparison of means from the one-way ANOVA results, shown in Table 4-4.

Table 4-4  
Consistency of Respirometry Runs using a Tukey-pairwise Comparison of O<sub>2</sub> Mean Totals (PG<sub>1000</sub> on Uncontaminated Soil, 288 hr point)

| RUN | MEAN  | HOMOGENEOUS<br>GROUPS |
|-----|-------|-----------------------|
| 2   | 44873 | I                     |
| 1   | 37551 | I                     |
| 5   | 37265 | I                     |
| 4   | 36837 | I                     |
| 3   | 35803 | I                     |

THERE ARE 2 GROUPS IN WHICH THE MEANS ARE NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

The comparison (Table 4-4) shows inconsistency in the mean O<sub>2</sub> consumption totals for Run-2, compared with the other respirometry run means. This supported the

removal of this data set. This infers that the laboratory procedure might have been compromised. The error might have been in the preparation of the propylene glycol solution. A higher concentration (greater than  $\geq 10,000$  mg/L) solution might have been prepared, thus causing the higher O<sub>2</sub> consumption totals.

In addition, Run-2 had been cut short at 288 hr point due to a power failure. This would have restricted the use/comparison of other respirometry runs/data that had operated for a full 336 hours in the research. This supported re-accomplishment of Run-2, and removing the old Run-2 data that was questionable.

After Run-2 was re-accomplished, a new statistical test was performed to check the consistency in laboratory procedures. The 288 hr time period for O<sub>2</sub> consumption totals were compared for outliers, using a Box and Whiskers plot. The plot showed no outliers. The residuals for each respirometry run were calculated and plotted on a Wilk-Shapiro/Rankit plot of residuals. The data appeared to have aptness from the Wilk-Shapiro statistic = .936 (acceptable).

An F-test value and P-value were provided from the one-way ANOVA results. The degrees of freedom were calculated and the F-critical value was determined. The results of the tests are summarized in Table 4-5.

Table 4-5  
One-way ANOVA results for PG<sub>1000</sub> on Uncontaminated Soil (288 hr point)

| Test                            | Testing Values<br>(Devore, 709) | Test Results<br>STATISTIX 4.1 | Results                |
|---------------------------------|---------------------------------|-------------------------------|------------------------|
| $f^* \geq F_{crit}$ Reject Null | $F_{crit} = 2.87$               | $f^* = 2.75$                  | Do not reject the Null |
| $P \leq \alpha$ Reject Null     | $\alpha = 0.05$                 | $P = 0.0649$                  | Do not reject the Null |

See Appendix M, page M-8 for results

The null hypothesis was not rejected, thus stating the propylene glycol soil treatment/runs have shown consistency. This prompted the completion of a Tukey-pairwise comparison of means from the one-way ANOVA results as shown in Table 4-6.

Table 4-6  
Consistency of Respirometry Runs using a Tukey-pairwise Comparison of O<sub>2</sub> Mean Totals (PG<sub>1000</sub> on Uncontaminated Soil, 288 hr point)  
(Run-2, re-accomplished and included)

| <u>RUN</u>  | <u>MEAN</u> | <u>HOMOGENEOUS<br/>GROUPS</u> |
|---|-------------|-------------------------------|
| 1   | 37551       | I                             |
| 5   | 37265       | I                             |
| 4   | 36837       | I                             |
| 2   | 36205       | I                             |
| 3   | 35803       | I                             |
| THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES<br>AMONG THE MEANS. |             |                               |

The incorporation of the new Run-2 data set has shown no significant difference amongst all the data sets (respirometry runs).

#### 4.2.3 Summary of Respirometry Data for Repeatability and Consistency

Overall, the comparison of O<sub>2</sub> results for all 2400+ respirometer run hrs (48,000+ microcosm hrs) showed consistency. This consistency is found in the comparison of background soil respiration and other similar treatments that were used throughout all six respirometry runs performed. Repeatability has definitely improved by following the recommendations of Johnson (1997) and O'Malley (1997). Other experiments by Thomas (1996), Totten (1995), and Baker (1995) also confirm the precision and accuracy of this particular respirometer.

### **4.3 Biodegradation Analysis of Respirometry Data (Phase-one)**

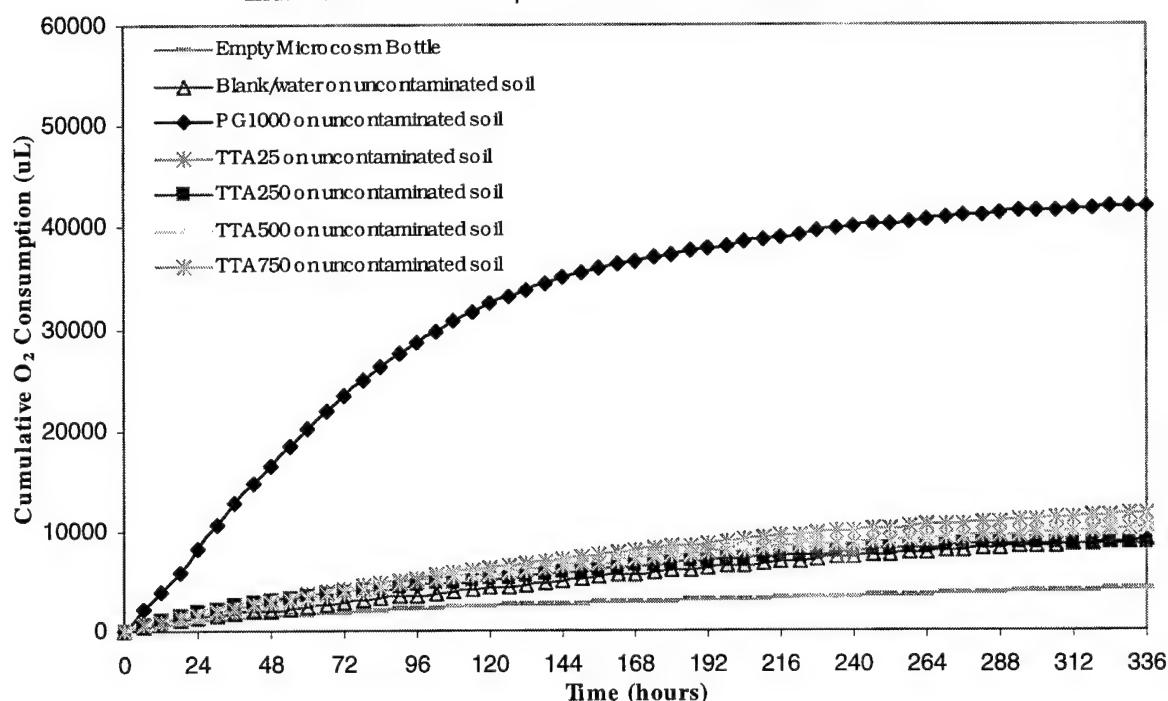
Respirometry work in phase-one used uncontaminated soils (unconditioned microorganisms). The uncontaminated media allowed measurements of microorganisms' initial response to the ADF's chemical components. The statistical tests were designed to determine if any effect (inhibition, biodegradation, or no effect) of O<sub>2</sub> consumption totals occurred due to the individual and combined ADF chemical treatments on soil. The procedures for statistical testing of individual ADF component treatments are summarized in Appendix F, and the combined ADF component treatments are summarized in Appendix G.

Biodegradation was measured through O<sub>2</sub> consumption and CO<sub>2</sub> production. Consumption and production activities were measured by recording accumulated totals (μL) and rates (μL/hr). CO<sub>2</sub> production mirrored O<sub>2</sub> consumption, consequently only O<sub>2</sub> data was analyzed. A representative collection of all plotted forms (μL and μL/hr) of O<sub>2</sub> and CO<sub>2</sub> data are found in Appendix E for respirometry Run-1 (see Figures E-1 through E-5).

#### **4.3.1 Analysis of Individual ADF Component Treatments on Uncontaminated Soil**

Figure 4-1 plots cumulative O<sub>2</sub> consumption measurements for the individual ADF chemical treatments on uncontaminated soil for phase-one. All ADF treatment lines depicted in the figure are an average of five microcosms and blank treatment lines are an average of three microcosms. Refer to Appendix E for the original data from respirometry runs (Run-1, Run-2, Run-3, and Run-5) related to Figure 4-1.

Figure 4-1  
Cumulative O<sub>2</sub> Consumption (μL) for  
Individual ADF Components on Uncontaminated Soil



Note: legend designation TTA25 (or others) refers to TTA<sub>25</sub> or 25 mg/kg tolyltriazole

Figure 4-1 demonstrated a higher cumulative O<sub>2</sub> consumption for propylene glycol compared to any of the tolyltriazole concentrations on soil. The figure also demonstrated when TTA<sub>25</sub>, TTA<sub>250</sub>, TTA<sub>500</sub>, or TTA<sub>750</sub> were placed on uncontaminated soil, the respiration totals were about the same as the blank treatment on uncontaminated soil.

The respirometry data for the rate of O<sub>2</sub> consumption was assembled from all the phase-one runs (Run-1, Run-2, Run-3, and Run-5) in Figure 4-2. All ADF treatment lines depicted in the figure are an average of five microcosms and the blank treatment lines are an average of three microcosms. Appendix E contains original respirometry runs related to Figure 4-2.



Figure 4-2  
Rate of O<sub>2</sub> Consumption (μL/hr) for Individual ADF Components  
on Uncontaminated Soil

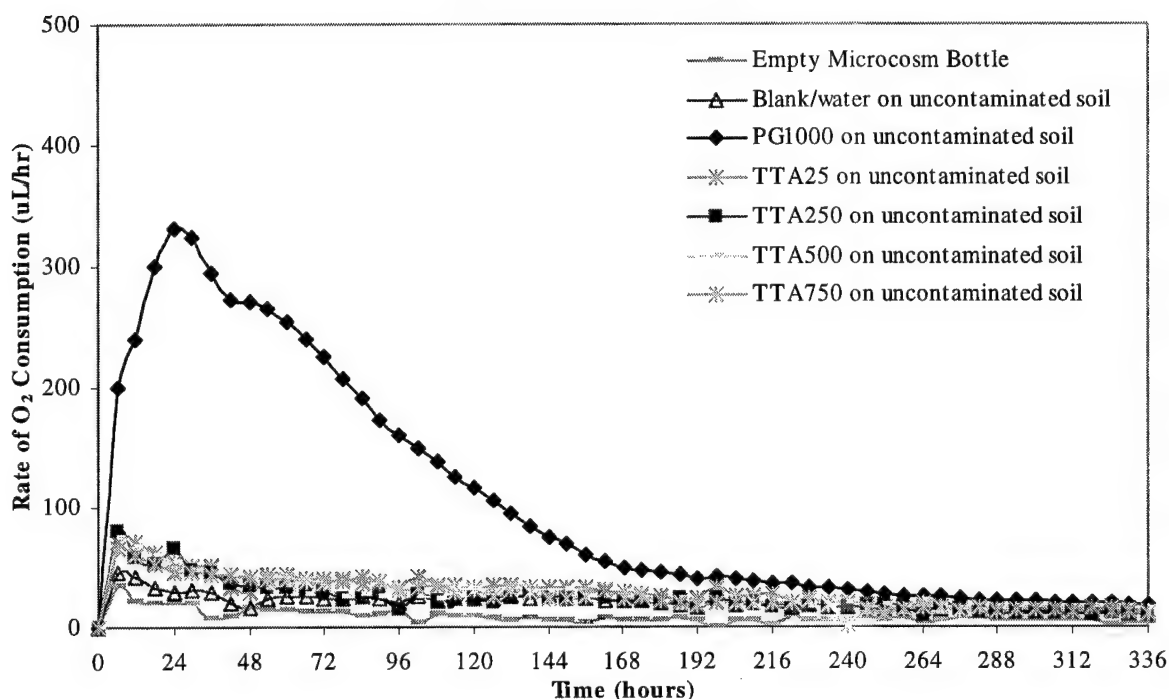


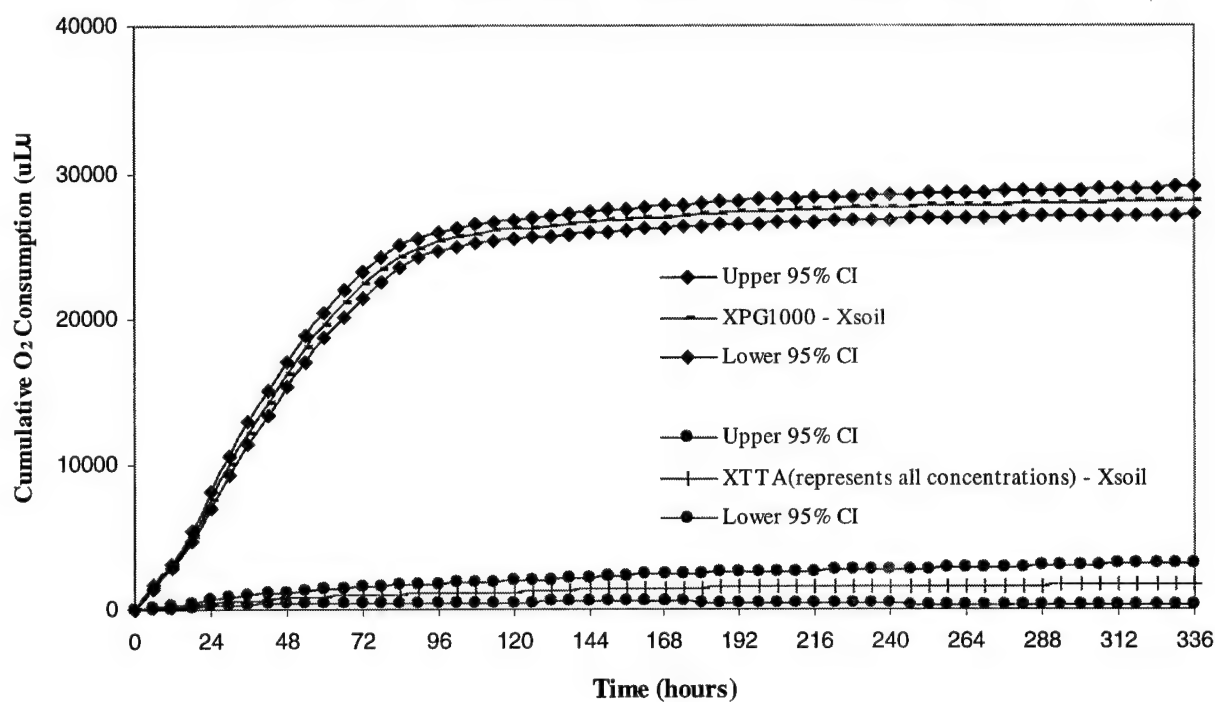
Figure 4-2 demonstrated O<sub>2</sub> consumption for PG<sub>1000</sub> had returned to blank soil treatment levels after the 264 – 336 hr point, while the TTA<sub>25-1000</sub> treatments were similar to blank soil respiration activity.

Statistical tests were then applied to the cumulative O<sub>2</sub> consumption totals to determine if the individual ADF components (PG alone or TTA alone) were greater than the blank soil treatment. The statistical tests were followed from Johnson's (1997) approach. The null hypothesis was that there was no effect on the O<sub>2</sub> consumption due to the contaminant addition compared to O<sub>2</sub> consumption of blank soil. Biodegradation was supported when there was a significant difference in the O<sub>2</sub> consumption for chemical treatment on soil against the blank treatment on soil [Johnson, 4-30]. The evaluation of biodegradation, inhibition, or no effect was produced through a two-tailed t-test, with a

significance level of  $\alpha = 0.05$ , at each of the 6 hour sampling intervals over the entire respirometry period. The results are found in Table F-1 through Table F-5.

A 95% CI was developed from the t-test results to visually depict the size of the difference in the O<sub>2</sub> consumption effects. If the CI hooked the zero line of the y-axis, then the null hypothesis was supported. If the lower CI was above the zero line of the y-axis, then significant O<sub>2</sub> consumption (biodegradation) was supported. While if the upper CI was the zero line of the y-axis then inhibition was supported. Figure 4-3 summarizes the 95% CI results found in Appendix F.

Figure 4-3  
Statistical Tests on Cumulative O<sub>2</sub> Consumption Totals (μL)  
for Individual ADF Chemical Components on Uncontaminated Soil



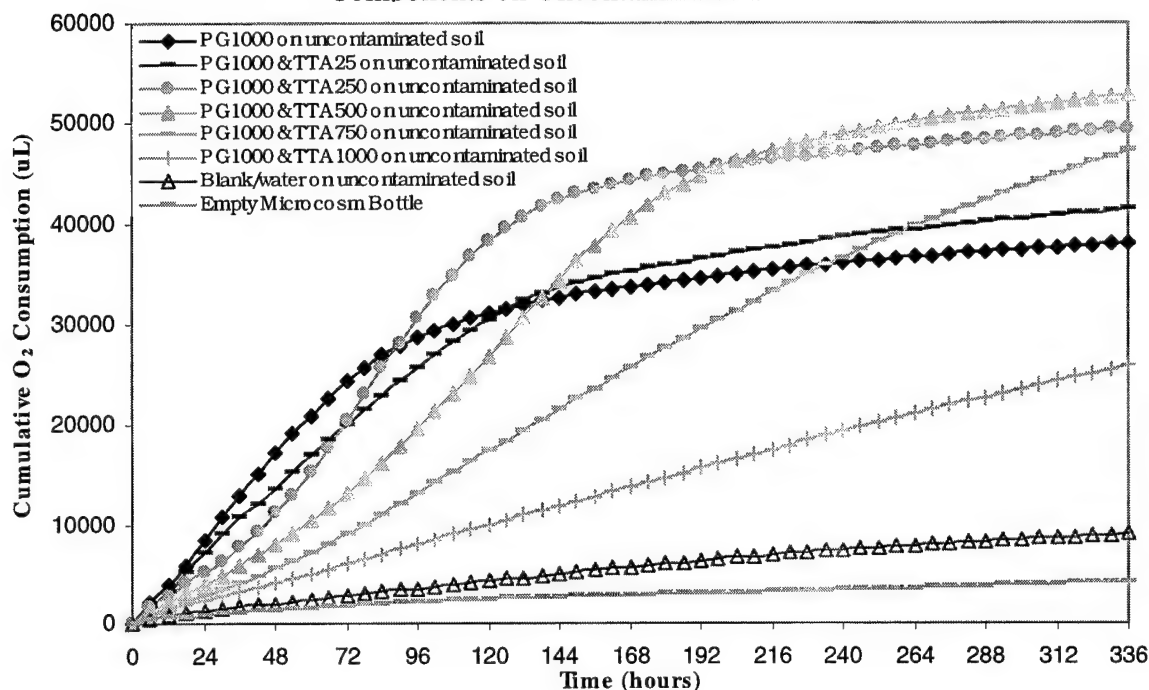
Note: Appendix F contains data referenced in Figure 4-3

The results showed that PG<sub>1000</sub> 95% CI (top lines) did not hook the zero line of the y-axis. Therefore the 95% CI indicates PG<sub>1000</sub> does consume O<sub>2</sub> above blank soil levels. This supports the potential biodegradation of propylene glycol alone in soil. A representative tolyltriazole CI was developed to represent the TTA<sub>25-750</sub> CI's (due to the overlap of the lines) and to establish a reference for the PG<sub>1000</sub> CI. The TTA<sub>25</sub> and TTA<sub>250</sub> 95% CI did hook the zero line of the y-axis, indicating no significant difference (no effect) in O<sub>2</sub> consumption occurred. However, there was additional O<sub>2</sub> consumption compared with blank soil respiration for TTA<sub>500</sub> and TTA<sub>750</sub>. This indicated some potential biodegradation of tolyltriazole alone in soil.

#### **4.3.2 Analysis of Combined ADF Component Treatments on Uncontaminated Soil**

Varied concentrations of tolyltriazole (25 – 1,000 mg/kg) were combined with a fixed concentration of propylene glycol (1,000 mg/kg) to determine if there were any effects on O<sub>2</sub> consumption (biodegradation). Figure 4-4 combines cumulative O<sub>2</sub> consumption measurements from all phase-one respirometry runs (Run-1, Run-2, Run-3, and Run-5). The ADF treatment lines depicted in Figure 4-4 are an average of five microcosms and the blank treatment lines are an average of three microcosms. Appendix E contains original respirometry runs related to Figure 4-4.

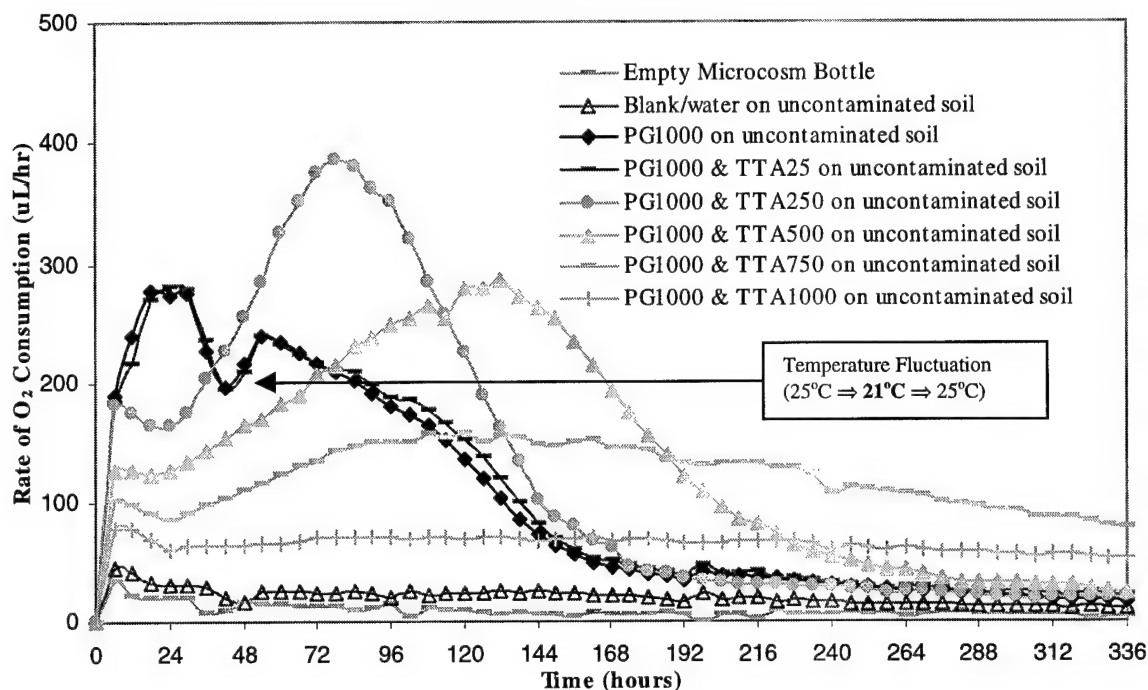
Figure 4-4  
Cumulative O<sub>2</sub> Consumption (μL) for Combined ADF Chemical  
Components on Uncontaminated Soil



The data in Figure 4-4 above, demonstrated that for mixtures of increasing TTA<sub>25⇒750</sub> with a fixed PG<sub>1000</sub>, the total accumulated O<sub>2</sub> consumption totals (336 hr point) increased compared to the a PG<sub>1000</sub> only treatment on soil. Figure 4-4 also demonstrated that the PG<sub>1000</sub> & TTA<sub>1000</sub> consumption totals were lower then PG<sub>1000</sub> only treatment on soil, due mainly to the reduced respiration activity seen in the rates of O<sub>2</sub> consumption.

Figure 4-5A and Figure 4-5B depicts the rate of O<sub>2</sub> consumption for the combined ADF components on uncontaminated soil from all the phase-one respirometry data. The plot lines in Figure 4-5A used an average of five microcosms for each ADF treatment, and three microcosms for the blank soil treatment.

Figure 4-5A  
Rate of O<sub>2</sub> Consumption (μL/hr) for Combined ADF Components  
on Uncontaminated Soil

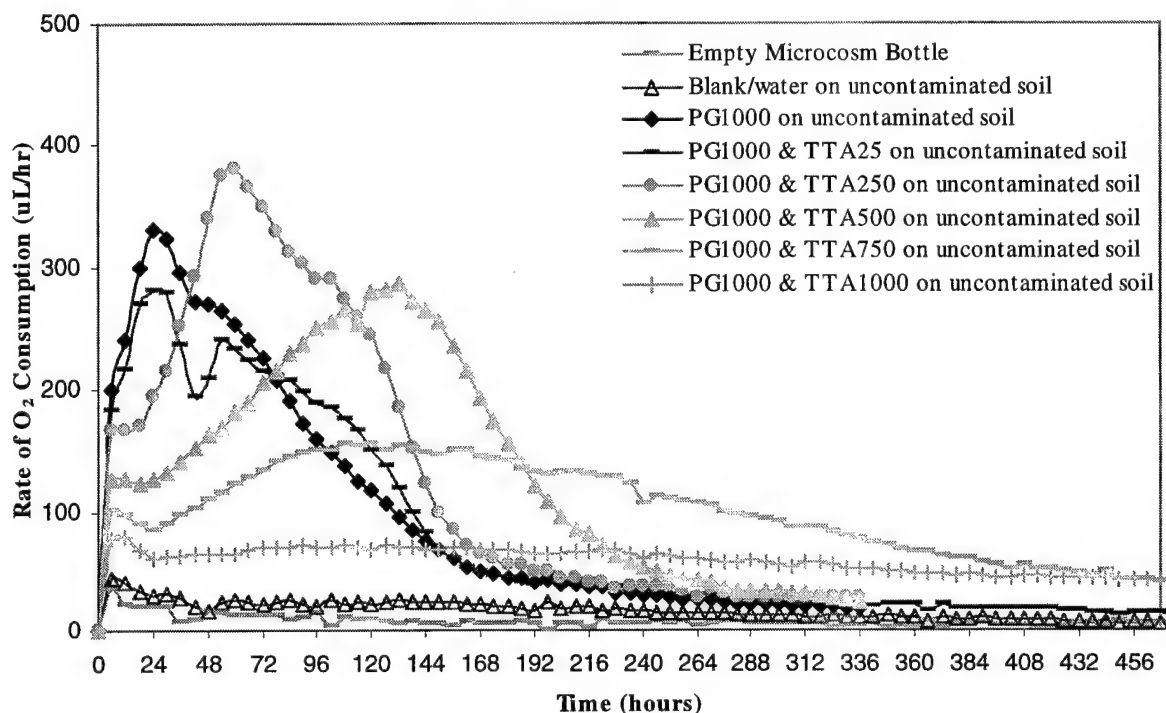


**Note:** The temperature fluctuation (PG<sub>1000</sub> & TTA<sub>25</sub>) reduced respiration activity for a limited time.

In Figure 4-5A, the PG<sub>1000</sub> and the PG<sub>1000</sub> & TTA<sub>25</sub> data lines were produced strictly from Run-1 data. The importance of this detail was to depict the minimal difference in the rates of O<sub>2</sub> consumption for the two treatments (PG<sub>1000</sub> and PG<sub>1000</sub> & TTA<sub>25</sub>).

In Figure 4-5B, an average of 15 microcosms (Run-1, Run-2, and Run-3) were used to depict the PG<sub>1000</sub> plot line, along with five microcosms for the other ADF treatments, and three microcosms for blank soil treatments. The time scale of the y-axis was also extended from 336 hrs to 468 hours. The longer time period enhanced the depiction of PG<sub>1000</sub> & TTA<sub>750</sub> and PG<sub>1000</sub> & TTA<sub>1000</sub> slowed rate of O<sub>2</sub> consumption.

Figure 4-5B  
Rate of O<sub>2</sub> Consumption (μL/hr) for Combined ADF Components  
on Uncontaminated Soil



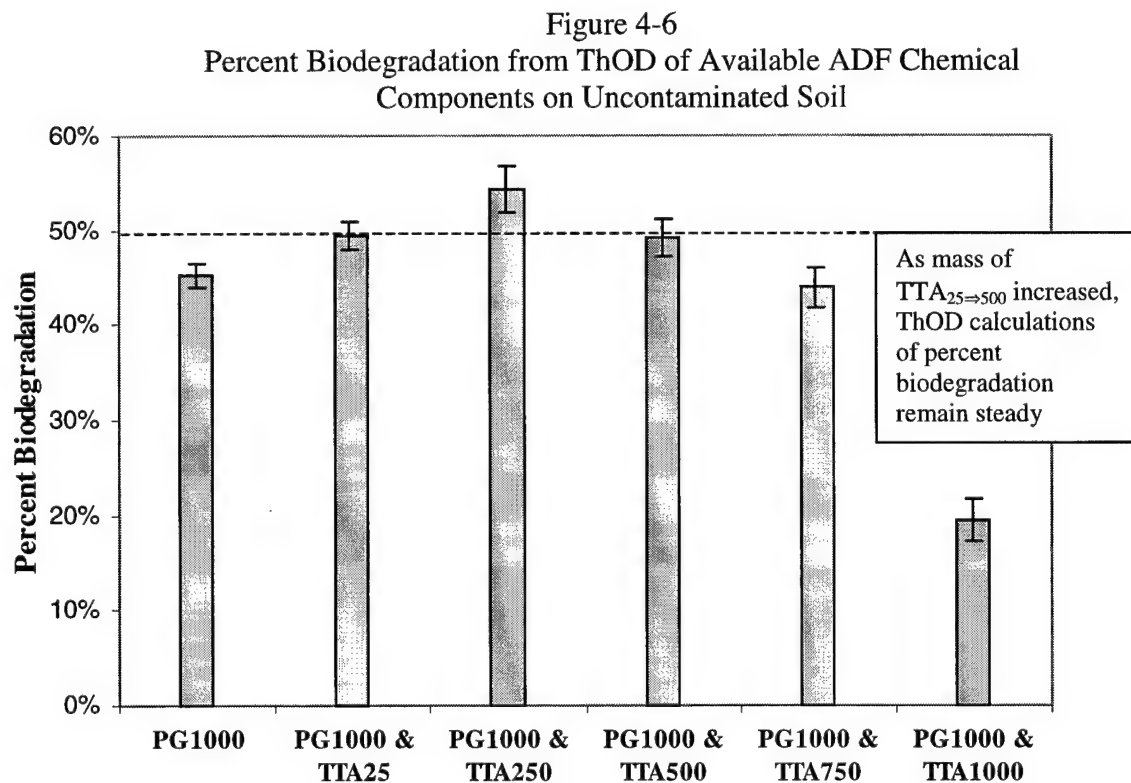
Both Figures 4-5A and Figure 4-5B demonstrated the slowing rate of O<sub>2</sub> consumption with the increasing concentration of TTA<sub>25⇒1000</sub> combined with PG<sub>1000</sub>. Even at the 468 hr point, the rate of O<sub>2</sub> consumption for the mixture of PG<sub>1000</sub> & TTA<sub>750</sub> and PG<sub>1000</sub> & TTA<sub>1000</sub> had not returned to the rate of O<sub>2</sub> consumption rate for blank soil.

ThOD equations for propylene glycol and tolyltriazole (section 2.3.5 and 2.3.7, respectively) were then applied to the observed effect (respirometry data) of increased O<sub>2</sub> consumption due to the increased mass TTA<sub>25⇒1000</sub> with a fixed mass of PG<sub>1000</sub> (Figure 4-5). The focus was on whether the apparent increase in O<sub>2</sub> consumption was proportional/correlated to the ThOD of ADF chemicals potential biodegradation in soil (PG<sub>1000</sub> & TTA<sub>25⇒1000</sub>). The “total” ThOD was calculated for the available mass of ADF

chemicals in the uncontaminated soil. The “total” ThOD results were then converted from mass (mg) O<sub>2</sub> to volume (μL) O<sub>2</sub>, using the Ideal Gas Law.

The “actual” O<sub>2</sub> consumption totals (μL) were collected from the various treatments (PG & TTA) where the rate of O<sub>2</sub> consumption had returned to blank soil respiration rates, typically around the 336 – 468 hour point. The term “actual” O<sub>2</sub> consumption total equals the O<sub>2</sub> consumption total of the ADF soil treatment minus the O<sub>2</sub> consumption total of the blank soil treatment.

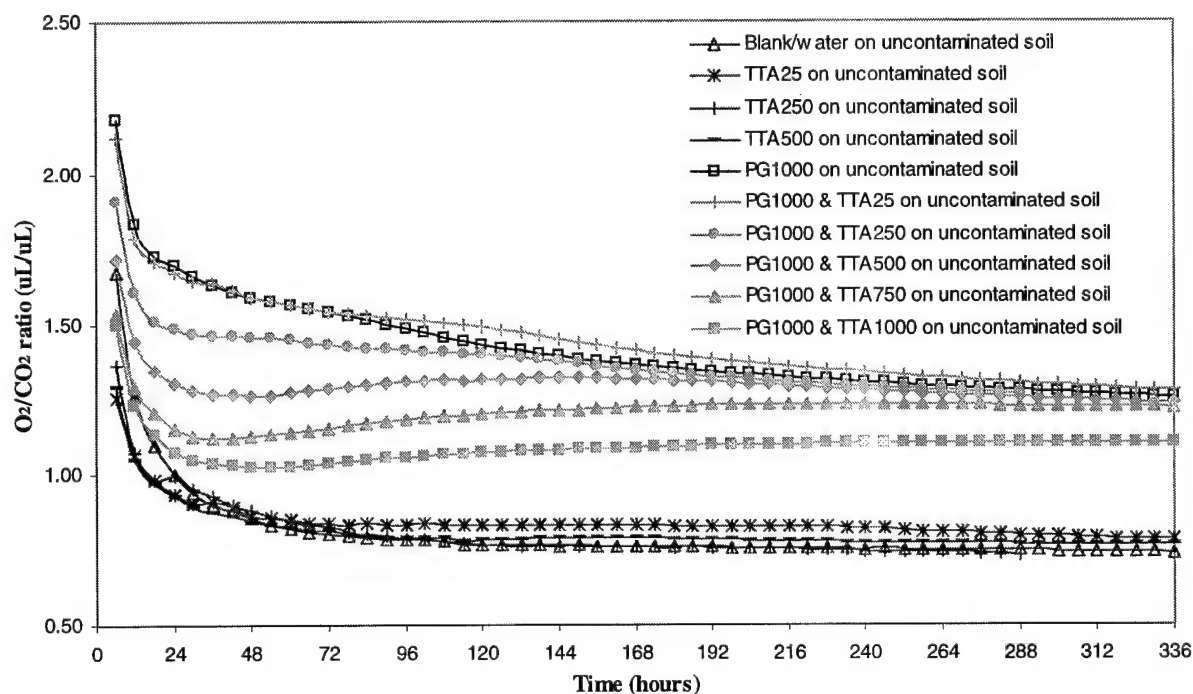
A percent biodegradation for available ADF components in soil was then calculated from the “actual” O<sub>2</sub> consumption total (μL) divided by the “total” ThOD (μL). Appendix K contains the data and calculations for the percent biodegradation shown in Figure 4-6.



The column graph demonstrated an approximately steady biodegradation percent (~50%) for a varied mass of TTA<sub>25</sub>⇒1000 with a fixed mass of PG<sub>1000</sub> in soil. PG<sub>1000</sub> & TTA<sub>750</sub> might also have achieved 50% biodegradation if the O<sub>2</sub> respiration activity had returned to blank soil respiration activity (uncompleted O<sub>2</sub> consumption). Note, the ThOD calculations for the percent biodegradation represent microbial respiration/activity for degrading the food source in an aerobic environment.

Figure 4-7 summarizes all of the respiration exchange ratios (RER's = O<sub>2</sub>/CO<sub>2</sub> in units of  $\mu\text{L}/\mu\text{L}$ ) for all of the phase-one respirometry runs.

Figure 4-7  
O<sub>2</sub>/CO<sub>2</sub> Ratios for All Phase-one Data



Overall, as the concentration of tolyltriazole increased with propylene glycol in soil, the overall RER's became lower. Perhaps the lowering RER's with increasing tolyltriazole concentrations was correlated to the ThOD calculations. The RER's were



calculated from the stochemetric equation from the ThOD calculations for propylene glycol and tolyltriazole (section 2.3.5 and 2.3.7, respectively). The two different ThOD RER's for propylene glycol and tolyltriazole were weighted with the amount of available chemical in the soil (Table 4-7).

Table 4-7  
Weighted ThOD RER's from Available ADF Components in Soil Treatments

| ThOD ratio for O <sub>2</sub> /CO <sub>2</sub>   |              | Treatment(s) of Propylene Glycol and Tolyltriazole in Soil |  |   |  |  |  |
|--|--------------|--|--|---|--|--|--|
|  |              | PG <sub>1000</sub> & TTA <sub>0</sub>                      |  | PG <sub>1000</sub> & TTA <sub>500</sub> |  | PG <sub>1000</sub> & TTA <sub>1000</sub> |  |
| Table 2-1  | PG = 1.333   | 1000/1000 * 1.33   |  | 1000/1500 * 1.33                        |  | 1000/2000 * 1.33                         |  |
| Table 2-2  | TTA = 0.9285 | 0/1000*.929  |  | 500/1500*.929                           |  | 1000/2000 *.929                          |  |
| Averaged O <sub>2</sub> /CO <sub>2</sub> ratio = |              | 1.333  |  | 1.198                                   |  | 1.130                                    |  |

Thus, a decreasing ThOD RER's would occur as calculated in Table 4-7 and might support the decreasing RER's seen in Figure 4-7.

A statistical test was conducted to identify the change on microbial respiration activity due to the combined ADF chemical treatment (PG & TTA) compared to individual ADF components (PG alone and TTA alone) on uncontaminated soil. The null hypothesis stated there was no difference in O<sub>2</sub> consumption due to combined ADF components compared to the individual ADF components on uncontaminated soil. This determination was made using O<sub>2</sub> consumption totals of the contaminated soil (PG & TTA) against a linear combination of individual treatments (PG alone, TTA alone, and blank) on uncontaminated soil. Appendix G contains a visual explanation of this linear combination. A two-sample t-test was used to measure the difference of O<sub>2</sub> total means using a significance level of  $\alpha = 0.05$ . Figure 4-8 depicts the set-up of the O<sub>2</sub> means totals to perform the t-test in the upcoming CI results (Figure 4-9).

The t-test results were converted into a 95% CI for the entire respirometry run

period (336 hrs). The CI provided a visual depiction of the amount  $O_2$  increased or decreased due to the combined ADF components compared to the individual effects of the ADF components. The null hypothesis was based around the zero line of the y-axis. Appendix G contains a detailed layout of the statistical set-up, formulas, and Figures G-1 through G-5. Figure 4-8 overlaid three statistical tests ( $PG_{1000}$  &  $TTA_{25}$ ,  $PG_{1000}$  &  $TTA_{500}$ , and  $PG_{1000}$  &  $TTA_{750}$ ) to show the differences in  $O_2$  consumption effects from the combination of ADF components.

Figure 4-8  
Difference Between the Means ( $O_2$ ) using a 95% Confidence Interval for the Linear Combination of Tolytriazole and Propylene Glycol on Uncontaminated Soil

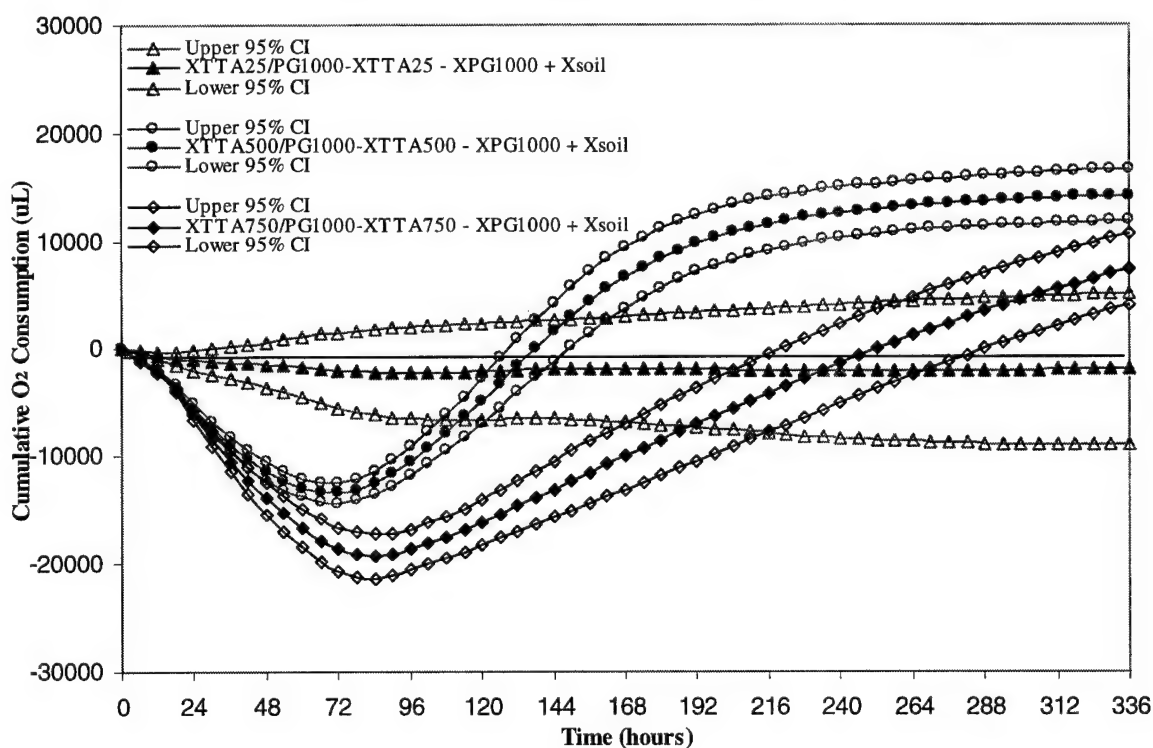


Figure 4-8 revealed no significant difference in  $O_2$  consumption when  $TTA_{25}$  was combined with  $PG_{1000}$ , since the 95% CI hooked the mean of the zero line of the y-axis (null hypothesis). The other comparison of  $PG_{1000}$  &  $TTA_{500}$  and  $PG_{1000}$  &  $TTA_{750}$

showed significant O<sub>2</sub> consumption effects due to the combination of propylene glycol and tolyltriazole in soil. The 95% CI reveals inhibition on O<sub>2</sub> consumption for the first 140 hrs, since PG<sub>1000</sub> & TTA<sub>500</sub> are below the zero, while PG<sub>1000</sub> & TTA<sub>750</sub> showed inhibition for the first 252 hrs.

These lags indicate unusual inhibition effects as the concentration of tolyltriazole increased with propylene glycol. As explained by Johnson (1997), the process of biodegradation usually begins after a lag period in which microorganisms are adjusting to the new contaminate(s) by producing needed enzymes. Populations that cannot handle a certain chemical and concentration might die off, and new populations will emerge in their place. The statistical test only confirms the unusual O<sub>2</sub> consumption activity.

#### **4.3.3 HPLC Analysis of Tolyltriazole Residual in Spent Soil**

HPLC analysis of tolyltriazole concentrations/residuals was performed before respirometry runs (without biodegradation pathway), and immediately after the respirometry runs (potential biodegradation pathway). The preparation of HPLC calibration curves for tolyltriazole detection is outlined in Appendix C. The methodology section (see page 3-18) contains the preparation of soil samples and the extraction processed used for measuring the tolyltriazole for HPLC analysis.

The HPLC calculations of percent degradation are found in Appendix H, and are summarized in Table 4-8A.

Table 4-8A  
Percentages of Tolyltriazole Residual Recovered

| Treatment                               | Percent of tolyltriazole residual measured through HPLC analysis |         |           |   |         |           |
|---|--|---------|-----------|---|---------|-----------|
|   | Before Respirometry Test (3 samples used)                        |         |           | After Respirometry Test (5 microcosms used) |         |           |
|   | Avg  | Std Dev | Reference | Avg   | Std Dev | Reference |
| TTA <sub>25</sub>                       | 99.79%   | 1.35%   | Table H-4 | 48.97%                                      | 5.05%   | Table H-5 |
| TTA <sub>250</sub>                      | 90.56%   | 0.33%   | Table H-4 | 81.51%                                      | 3.89%   | Table H-6 |
| TTA <sub>500</sub>                      | 95.15%   | 0.08%   | Table H-4 | No test performed                           | -----   | -----     |
| PG <sub>1000</sub> & TTA <sub>25</sub>  | 97.21%   | 1.17%   | Table H-4 | 40.17%                                      | 3.73%   | Table H-5 |
| PG <sub>1000</sub> & TTA <sub>250</sub> | 95.59%   | 0.17%   | Table H-4 | 73.43%                                      | 3.23%   | Table H-6 |
| PG <sub>1000</sub> & TTA <sub>500</sub> | 95.93%   | 0.12%   | Table H-4 | No test performed                           | -----   | -----     |

**Note:** No HPLC tests were performed on spent respirometry soil from Run-3 (TTA<sub>500</sub> and PG<sub>1000</sub> & TTA<sub>500</sub>) due to use in the phase-two experiments.

The tolyltriazole percent recovered before respirometry runs showed that the majority was recovered (90 – 99%), with or without the presence of propylene glycol, when immediately extracted from the soil. The results are not necessarily a good baseline to compare for potential biodegradation after the respirometry. There are too many degradation pathways to account for the loss of tolyltriazole (18 – 60%) when in contact with the soil (two weeks). These unknown degradation pathways were things such as the potential for strong absorption of the chemicals to the soil, physical change of the chemicals, or biotic reaction to the chemicals.

However, specific attention was placed on the additional degradation of tolyltriazole when in the presence of propylene glycol. This attention was supported by the respiration data, which had shown a larger O<sub>2</sub> consumption totals (μL) for the combination of propylene glycol and tolyltriazole compared to propylene glycol alone (as supported in Figure 4-6).

A pattern of additional degradation was observed for the mass of tolyltriazole when present with propylene glycol, as shown in Table 4-8B.

Table 4-8B  
Percentages of Tolyltriazole Residual Recovered

| Treatment                               | Percent of tolyltriazole residual<br>measured through HPLC analysis<br>After Respirometry Test (5 microcosms used) |         |
|---|--|---------|
|   | Avg  | Std Dev |
| TTA <sub>25</sub>                       | 48.97% ←   | 5.05%   |
| TTA <sub>250</sub>                      | 81.51% ←   | 3.89%   |
| TTA <sub>500</sub>                      | No test performed  | -----   |
| PG <sub>1000</sub> & TTA <sub>25</sub>  | 40.17% ←   | 3.73%   |
| PG <sub>1000</sub> & TTA <sub>250</sub> | 73.43% ←   | 3.23%   |
| PG <sub>1000</sub> & TTA <sub>500</sub> | No test performed  | -----   |

8.8% Δ ± Std Dev

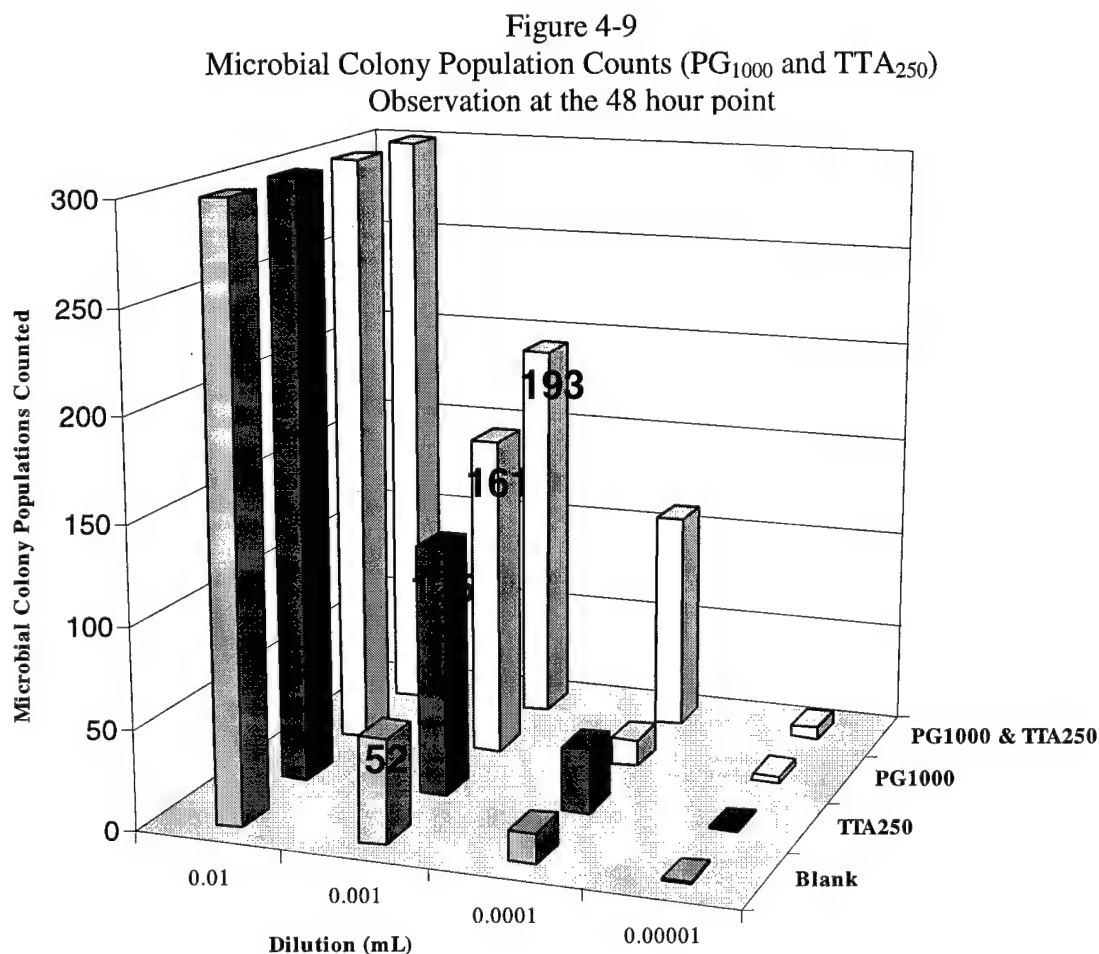
8.1% Δ ± Std Dev

A statistical test was performed on the HPLC data to see if these additional degradation percentages (8.8% and 8.1%) were similar for the two different tolyltriazole concentrations in the presence of propylene glycol, or undeterminable due to their standard deviations. A two-sample t-test of the differences was performed using a significance level of  $\alpha = 0.05$ . The null hypothesis was that the additional degradation percentages were similar in value for the two different treatments of TTA. The null was accepted, and the HPLC results supported a consistent percent (8.1 – 8.8%) of additional degradation for the varied mass of TTA<sub>25-250</sub> when in the presence of fixed mass of PG<sub>1000</sub>.

Kellner's (1999) results of sorption/desorption of tolyltriazole with this soil showed interesting results. Using a different technique for HPLC analysis, he identified that tolyltriazole appears to strongly sorb to the organic material of the (high-clay) soil (approximately 0.7 – 1.3 mg TTA/100 gm soil). He also performed a HPLC analysis on the spent soil from this experiment. The HPLC detection areas revealed another area peak, along with the two isomers peaks of tolyltriazole. This third peak area is considered to be a reduced form of the tolyltriazole chemical, as proposed in Figure 2-2.

#### 4.3.4 Analysis of Microbial Colony Plate Count Results

The microbial colony plate count test used spent soil from phase-one respirometry experiments. The visual results depict the influence ADF chemicals had upon microbial populations within the soil/chemical environment. Two chemical concentrations of tolyltriazole (250 mg/kg and 500 mg/kg) were tested and are shown in Figures 4-9 and Figure 4-10, respectively. Data can be found in Appendix I.

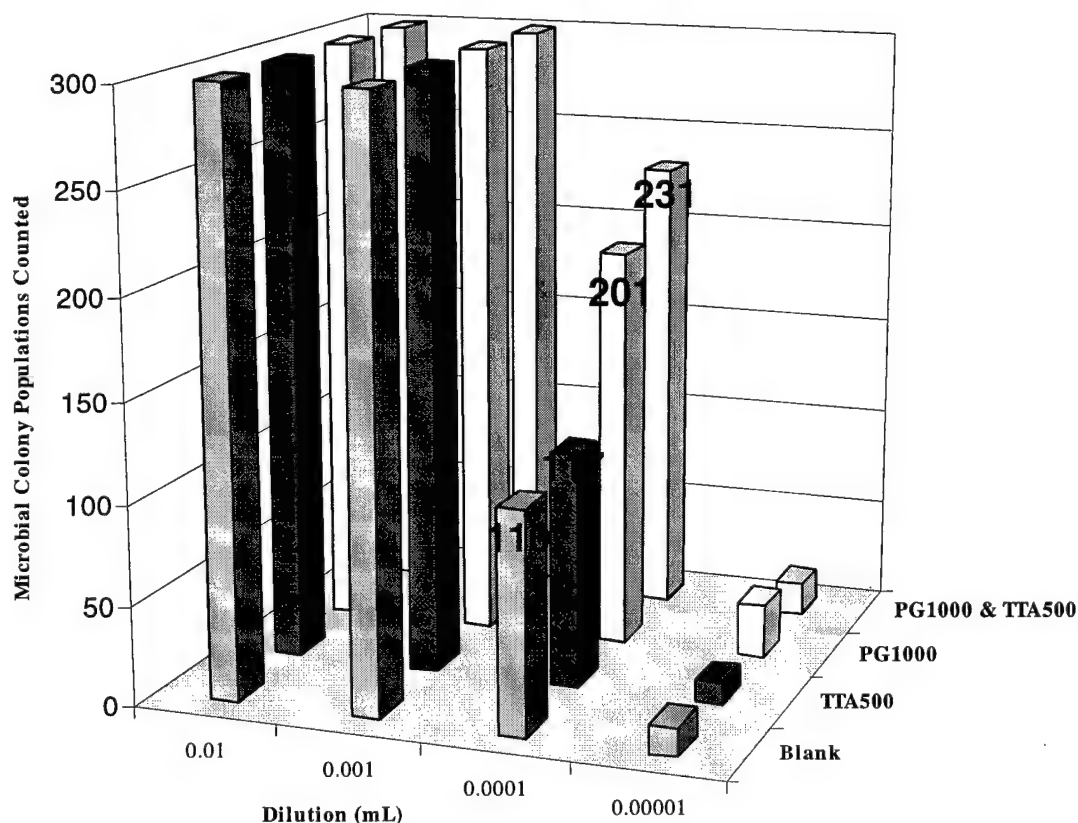


**Note:** Each column represents an average of three petri dishes, counted three times and averaged.

In Figure 4-9 above, the dilution range of 0.001 produced a range of 52 – 193 colonies. This range of colonies was within the acceptable range/limits of evaluation

(30 – 300) as described in *Standard Methods*. Uncontaminated soil (blank) was the base line for the population of microorganisms. The MCPC results showed that concentrations and combinations tested for PG<sub>1000</sub> and TTA<sub>250</sub> had no toxic effect on populations of microorganism in soil.

Figure 4-10  
Microbial Population Counts (PG<sub>1000</sub> and TTA<sub>500</sub>)  
Observation at the 48 hour point



**Note:** Each column represents an average of three petri dishes, counted three times and averaged.

In Figure 4-10 above, the dilution range of 0.0001 produced a range of 110 – 231 colonies. This range of colonies was within the acceptable range/limits for evaluation (30 – 300) as described in *Standard Methods*. Uncontaminated soil (blank) was the base line for the population of microorganisms. The MCPC results showed that

concentrations and combinations tested for PG<sub>1000</sub> and TTA<sub>500</sub> had no toxic effect on populations of microorganism in soil.

Both MCPC figures indicated that these concentrations and combinations of ADF components did not affect the populations of soil microorganisms.

#### **4.3.5 Analysis of Agar Well Diffusion Test Results**

The agar well diffusion test was performed with a propylene glycol concentration of 10,000 mg/L and tolyltriazole concentrations of 5,000 – 10,000 mg/L. Individual and combined mixtures of these ADF components were applied. The tests followed the methodology section 3.7. The visual data are located in Appendix J. The results indicated no toxic effects to microbial population growth around the agar well. This indicates no toxic effects from individual and combined ADF chemical components.

#### **4.4 Biodegradation Analysis of Respirometry Data (Phase-two)**

Phase-two of this research was designed to determine if application of PG<sub>1000</sub> on acclimated soil/microorganisms would produce different respiration activity. The expectation was increased biodegradation of materials, since microorganisms were acclimated to the chemicals. This would reduce lag time and increase the initial biodegradation rate of microbes.

Phase-two research also looked at the effects of residual tolyltriazole in soil. The comparison of acclimated soils (PG alone, TTA alone, and PG & TTA) new O<sub>2</sub> consumption rates after PG<sub>1000</sub> was applied. Figure 4-11 shows various rates of O<sub>2</sub> consumption for combined ADF components on acclimated soil.



Figure 4-11  
Rate of O<sub>2</sub> Consumption (μL/hr) for Propylene Glycol (1,000 mg/kg) on  
Uncontaminated Soil and Acclimated ADF Chemical Soils

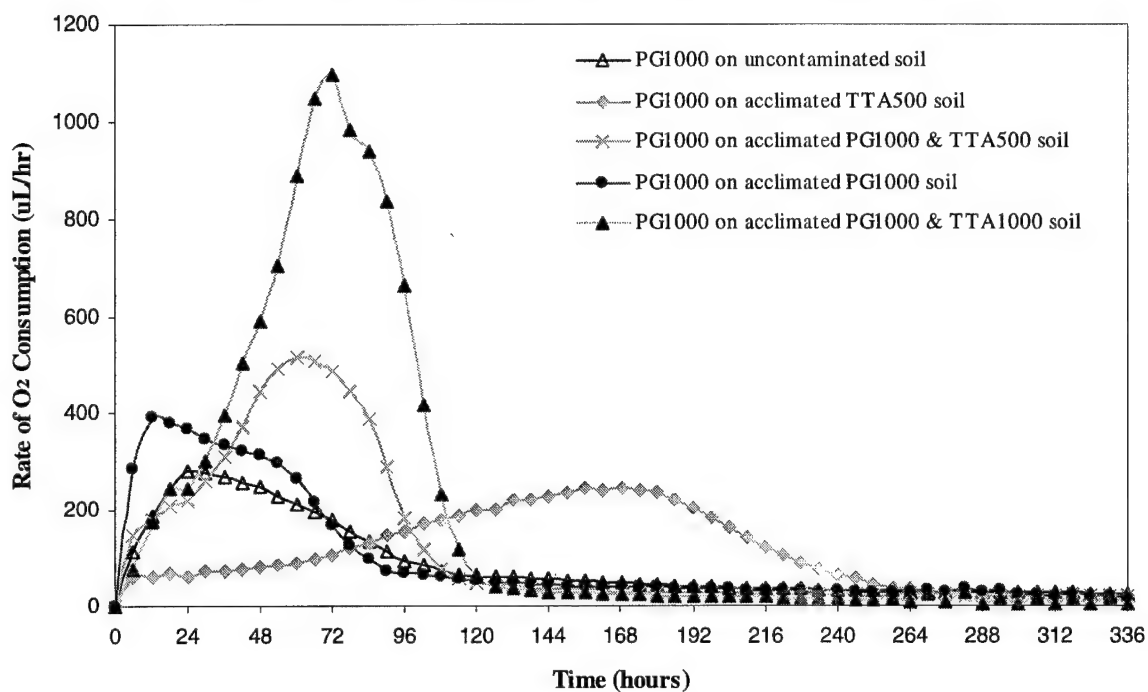
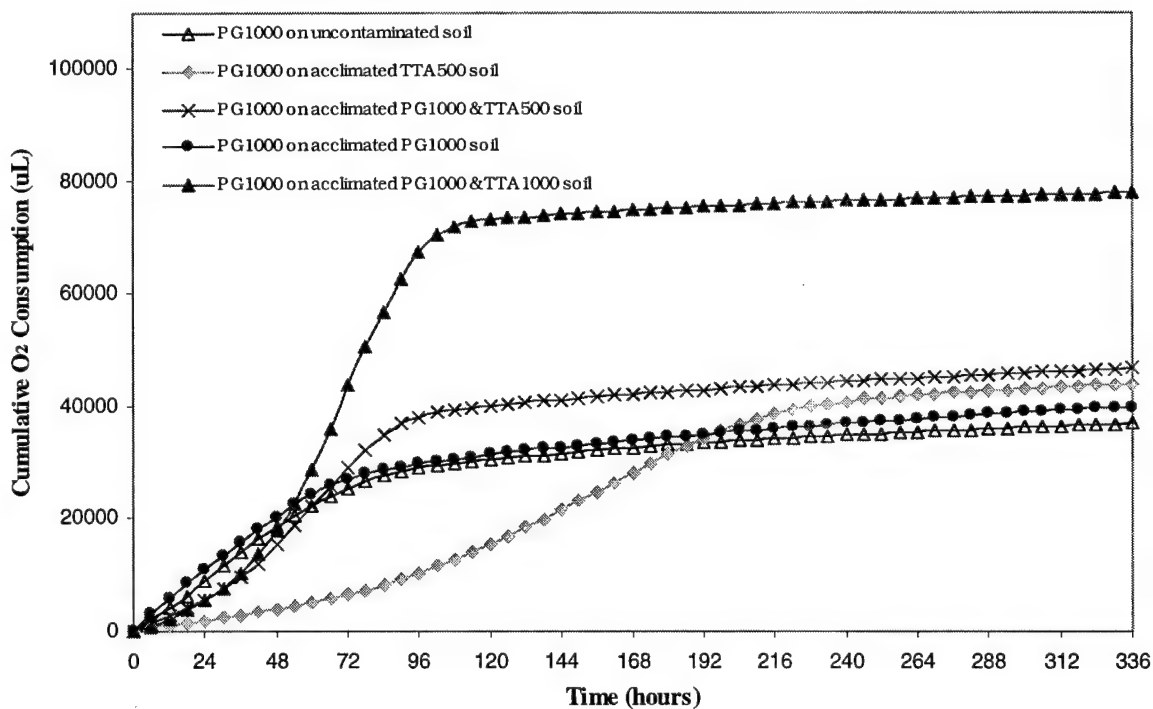


Figure 4-12  
Cumulative O<sub>2</sub> Consumption (μL) for Propylene Glycol (1,000 mg/kg) on  
Uncontaminated Soil and Acclimated ADF Chemical Soils



In Figure 4-11, an unexpectedly higher cumulative O<sub>2</sub> consumption total (~80K µL, at 336 hr point) was noticed, and a higher rate of O<sub>2</sub> consumption (Figure 4-12) was observed in the acclimated PG<sub>1000</sub> & TTA<sub>1000</sub> soil, after PG<sub>1000</sub> was applied. The reason might be residual propylene glycol slowed the rate of O<sub>2</sub> consumption from PG<sub>1000</sub> & TTA<sub>1000</sub> combination on uncontaminated soil (Figures 4-7).

There was another unexpected result for the two acclimated soils (TTA<sub>500</sub> and PG<sub>1000</sub> & TTA<sub>500</sub>) rates of O<sub>2</sub> consumption (Figure 4-12). There should have been no rate difference, if the tolyltriazole residuals from the phase-one soil treatments (PG<sub>1000</sub> & TTA<sub>500</sub> and TTA<sub>500</sub>) were equal (no loss to chemical, biological, and/or physical).

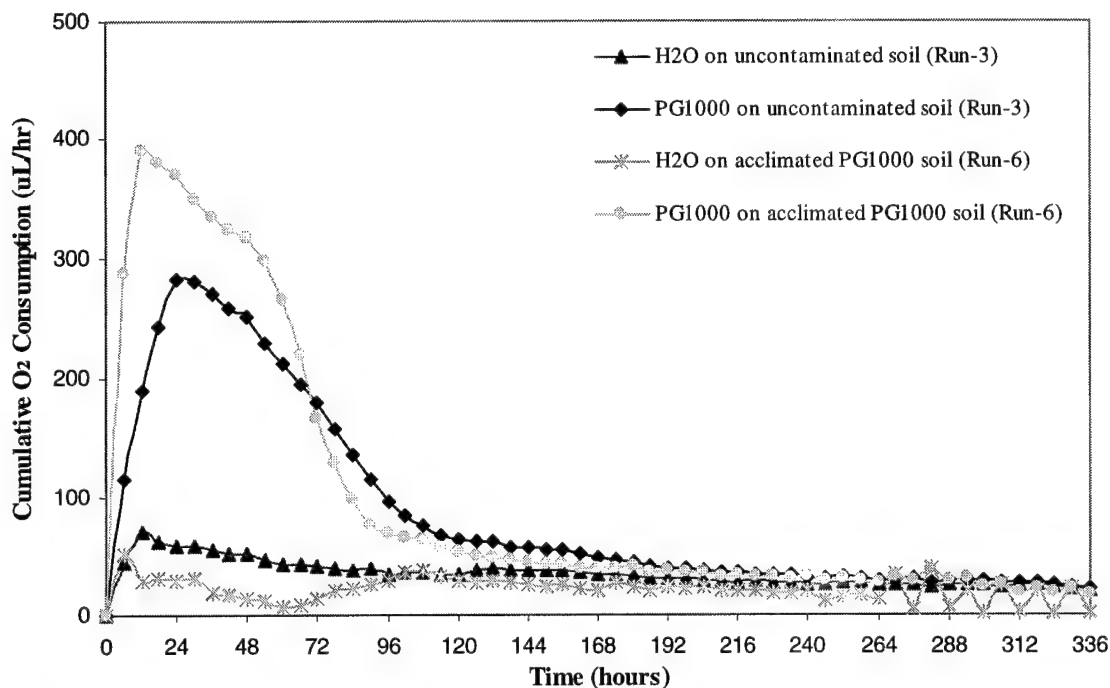
#### **4.5 Phase-one Compared to Phase-two Initial Biodegradation Rates**

Statistical testing was used to compare PG<sub>1000</sub> application on uncontaminated soil (phase-one data) versus PG<sub>1000</sub> re-application on PG<sub>1000</sub> acclimated soil. The specific focus was to determine if there were any effects in initial O<sub>2</sub> consumption rates (biodegradation) from unacclimated compared to acclimated microorganism.

The statistical test used a two-tailed t-test, with a significance level of  $\alpha = 0.05$ . The null hypothesis was stated as: There was no difference between initial O<sub>2</sub> consumption rates (initial biodegradation rates) from PG<sub>1000</sub> treatment on uncontaminated (phase-one) versus PG<sub>1000</sub> acclimated soil (phase-two).

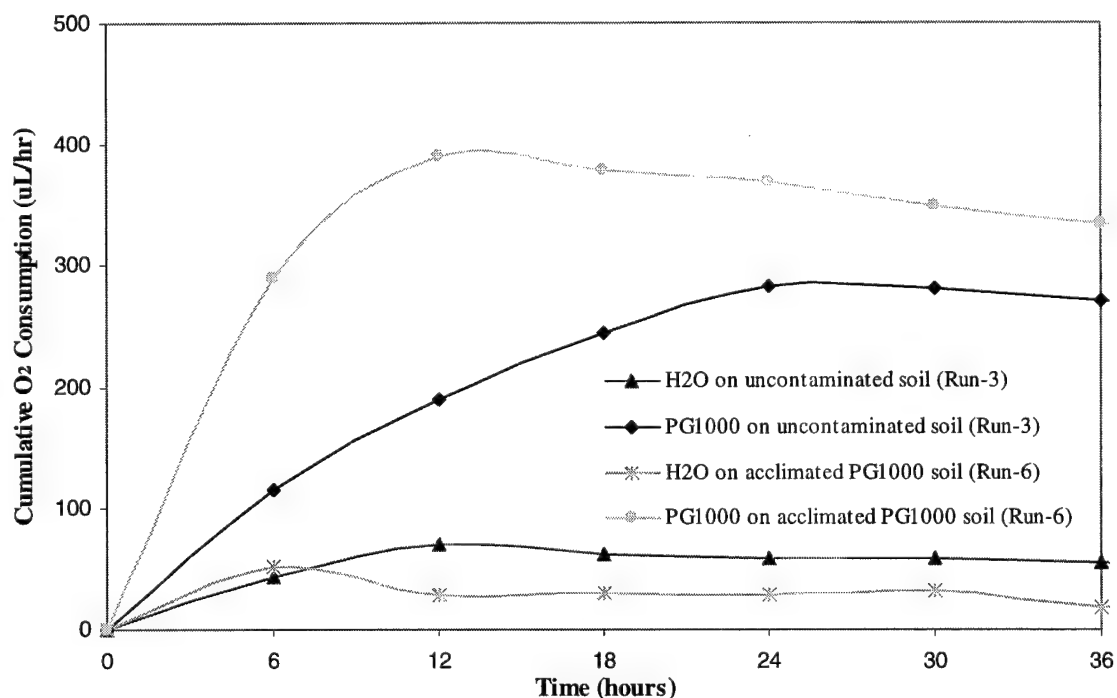
The biodegradation rates were generated from the ThOD calculations used in Appendix K. The maximum/initial biodegradation rates were visually determined by combining the applicable data from both phase-one and phase-two. Figure 4-13 combines data from Run-3 and Run-6.

Figure 4-13A  
Both Phases Rate of O<sub>2</sub> Consumption from Respirometry Data (336 hrs of Data)



Note: Maximum/initial rates of O<sub>2</sub> consumption were determined with in the 24 – 36 hr time period. Figure 4-13A was enlarged to provided a more useful graph (Figure 4-13B) for visual analysis.

Figure 4-13B  
Both Phases Initial Rate of O<sub>2</sub> Consumption from Respirometry Data (36 hrs of Data)



The first 24 hrs of cumulative O<sub>2</sub> consumption totals were processed using equations found in Appendix K. The calculations developed the initial biodegradation rates per mass of soil (mL/min/kg) for the two different O<sub>2</sub> consumption totals. The initial biodegradation rates were then statistically compared using the two-tailed t-test procedures explained in Appendix L. The results are summarized in Table 4-9 shown.

Table 4-9  
Statistical Test of Acclimated versus Uncontaminated Soils  
Initial Biodegradation Rates

| Test Statistic  | t-value | t-critical |                       |
|---|---------|------------|-----------------------|
| $-t_{crit} \leq t^* \leq t_{crit}$                      | $t^*$   | $t_{crit}$ | Reject H <sub>o</sub> |
| $t^*$ between $t_{crit}$ , do not reject H <sub>o</sub> | 27.52   | 2.78       | <b>Yes</b>            |

The null hypothesis was rejected; stating that there was a significant increase in initial biodegradation rates when PG<sub>1000</sub> was applied on acclimated soil (with PG<sub>1000</sub>) compared to the initial biodegradation rates of PG<sub>1000</sub> application on uncontaminated soil.

## V. Conclusions and Recommendations

### 5.1 Conclusions

The objective of this research was to study the effects on microbial degradation of ADF components in a (high-clay) soil environment. Previous studies have shown varied effects on microbial degradation of propylene glycol and tolyltriazole. The objective was to expand the research with varied concentrations to better understand microbial response to these chemicals.

Phase-one respirometry tests measured biodegradation effects of ADF chemicals upon uncontaminated clay soil. The ADF component propylene glycol (1,000 mg/kg) showed measurable O<sub>2</sub> consumption in soil compared to blank soil. The ADF component tolyltriazole (25 – 750 mg/kg) showed minimal O<sub>2</sub> consumption in soil compared to blank soil.

These ADF chemicals were combined to test the effects of tolyltriazole on the known O<sub>2</sub> consumption activity of propylene glycol in soil. Propylene glycol (1,000 mg/kg) mixed with different concentrations of tolyltriazole (25 – 1,000 mg/kg) showed varying respiration results. The rate of O<sub>2</sub> consumption slowed with increasing concentrations of (250 ⇒ 1,000 mg/kg) tolyltriazole with a fixed mass of (1,000 mg/kg) propylene glycol. Lower concentrations of (25 mg/kg) tolyltriazole with a fixed mass of (1,000 mg/kg) propylene glycol (similar to field conditions) showed little change in the rate of O<sub>2</sub> consumption. The higher concentrations of (750 – 1,000 mg/kg) tolyltriazole with a fixed mass of (1,000 mg/kg) propylene glycol had a significantly lower rate of O<sub>2</sub> consumption. Overall, as the (25 – 750 mg/kg) tolyltriazole increased with a fixed (1,000

mg/kg) propylene glycol, the O<sub>2</sub> consumption totals increased.

ThOD calculations for microbial degradation of these two components supported the idea of tolyltriazole's biodegradation with propylene glycol. In other words, as tolyltriazole increased in concentrations, a proportional (ThOD calculations = equation for microbial breakdown of chemicals) amount of O<sub>2</sub> consumption occurred. This supports the biodegradation/breakdown of tolyltriazole with propylene glycol.

The HPLC data could not demonstrate the biodegradation potential of tolyltriazole in soil, due to numerous degradation pathways (chemical, physical, and/or biotic). However, the potential for a biodegradation pathway was associated with the lower concentrations of (25 – 250 mg/kg) tolyltriazole when in the presence of (1,000 mg/kg) propylene glycol. HPLC results showed additional degradation (8.1 – 8.8%) of tolyltriazole mass occurred when in the presence of a fixed amount of propylene glycol. This supported the increased O<sub>2</sub> consumption totals as the mass of tolyltriazole increased when in the presence of a fixed mass of propylene glycol.

In conclusion of phase-one results, the respirometry data would imply that (1,000 mg/kg) propylene glycol biodegrades alone in soil, while little to no biodegradation occurs for (25 – 750 mg/kg) tolyltriazole alone in soil. Respirometry and HPLC data implies some potential biodegradation of (25 – 500 mg/kg) tolyltriazole mass in the presence of (1,000 mg/kg) propylene glycol.

The MCPC test revealed that the populations of microbes, acclimated in soil contaminated with ADF components, appeared to stay consistent or higher than microbial populations in uncontaminated soil. The AWDT revealed that microbes would grow upon solutions of ADF components (TTA and/or PG) without inhibition. Both of the toxicity

tests showed no adverse effects upon microorganisms in soil from tolyltriazole and propylene glycol chemicals.

Phase-two of this study evaluated biodegradation when propylene glycol was re-applied to acclimated soil from the phase-one study. Focus was on the comparison of (1,000 mg/kg) propylene glycol initial rate of biodegradation (O<sub>2</sub> consumption) on uncontaminated soil and acclimated soil (with propylene glycol only). Table 5-1 summarizes the initial biodegradation rates calculated from the respirometry data.

Table 5-1  
Initial Biodegradation Rates for Propylene Glycol (1,000 mg/kg) Application on  
Propylene Glycol Acclimated Soil and Uncontaminated Clay Soil

| Propylene Glycol (1,000 mg/kg) Application |                                      |
|--|--------------------------------------|
| Uncontaminated Soil                        | Acclimated Soil                      |
| Biodegradation Rate (mL/day/kg soil)       | Biodegradation Rate (mL/day/kg soil) |
| 107.41                                     | 148.81                               |

Statistical tests supported the idea that when propylene glycol (1,000 mg/kg) was applied to both acclimated and uncontaminated soil, the initial biodegradation rate of acclimated soil was significantly faster than the rate for uncontaminated soil.

## 5.2 Improvements

### 5.2.1 Use of HPLC with Indirect UV Detection

The use of HPLC methods with indirect UV detection has been established using derivatization [Massaccesi, 1992]. This could be applied to residual propylene glycol in the soil.

### **5.2.2 Modifying the HPLC with Refractive Index Detection**

The modification of the HPLC with refractive index detection equipment is another approach for propylene glycol detection in the aqueous phase. The protocols and detection limits are established (Nitschke *et al.*, 1994) for this refractive index detection. This could provide a mass accounting of propylene glycol after respirometry research.

### **5.2.3 Gas Chromatography with Flame Ionization Detection**

The use of Gas Chromatography with Flame Ionization Detection (GC/FID) has been established by methods used in Kaplan *et al.* (1982) research on glycol. These methods of GC/FID could be applied to the residual propylene glycol in soil.

### **5.2.4 Modifying the Respirometer**

The addition of ammonia and methane detection equipment to the respirometer would provide possible investigations in anaerobic conditions. This is one of the proposed pathways for the biodegradation of tolyltriazole.

## **5.3 Follow-on Research**

### **5.3.1 Investigating other components in ADFs**

There are several other additives within the ADFs. The biodegradation potential of one or more of these additives with propylene glycol would reveal other interaction effects on biodegradation potential.



### 5.3.2 Multiple Recontamination of ADF Components on Soil

A possible area of focus would be multiple applications of ADF components on soil. Developing an overall biodegradation rate trend from the various recontamination phases could be the focus question. The research could develop a long-term trend of increased/steady-state/decreased biodegradation rates for the ADF components. Then development and optimization of ADF application cycles on soil could be approached. Some examples might be the following:

1. (PG<sub>1000</sub> & TTA<sub>10</sub>) then (PG<sub>1000</sub> & TTA<sub>10</sub>) then (de-ionized water) → repeat cycle, or
2. (PG<sub>1000</sub> & TTA<sub>10</sub>) then (de-ionized water) then (PG<sub>1000</sub> & TTA<sub>10</sub>) → repeat cycle, or
3. (PG<sub>1000</sub> & TTA<sub>10</sub>) then (PG<sub>1000</sub>) then (PG<sub>1000</sub> & TTA<sub>10</sub>) → repeat cycle

### 5.3.3 Field Tests of ADF Component Biodegradation

Field testing ADF component degradation (bio and chemical) in an *in-situ* environment. Through establishment of a test area, application of different concentration and combinations of ADF components could be studied. HPLC or GC/FID analysis of residual concentrations might be applied to determine field versus laboratory results.

**Appendix A:**  
**Independent Soil Analysis**

AFIT/ENV/Charles A Bleckmann  
2950 P Street  
Wright-Patterson AFB OH 45440

Colorado State University  
Soil, Water and Plant Testing Laboratory  
Natural & Environmental Sciences Bldg - A319  
Fort Collins, CO 80523

DATE RECEIVED: 12-14-1998  
DATE PARTIAL REPORTED: 01-22-1999  
DATE REPORTED: 02-16-1999

(970) 491-5061 FAX: 491-2930

BILLING:

# RESEARCH SOIL ANALYSIS

8-Dec-98

| Lab # | Sample ID # | -----paste----- |  | EC mmhos/cm | Lime Estimate | % OM | -----AB-DTPA Extract----- |     |      |      |      |      |      |  |
|-------|-------------|-----------------|--|-------------|---------------|------|---------------------------|-----|------|------|------|------|------|--|
|       |             | pH              |  |             |               |      | NO <sub>3</sub> -N        | P   | K    | Zn   | Fe   | Mn   | Cu   |  |
| R3392 | Aft # 1     | 7.8             |  | 1.2         | Medium        | 2.7  | 3.3                       | 5.3 | 91.7 | 2.44 | 50.0 | 2.89 | 2.93 |  |
| R3393 | Aft # 2     | 7.7             |  | 0.9         | Medium        | 2.9  | 6.2                       | 4.9 | 92.0 | 2.63 | 51.7 | 2.76 | 3.15 |  |
| R3394 | Aft # 3     | 7.8             |  | 0.8         | Medium        | 3.0  | 6.2                       | 5.6 | 99.2 | 2.70 | 50.2 | 2.63 | 2.89 |  |

| Lab # | Sample ID # | %    |      |     | Clay | Texture | % |
|-------|-------------|------|------|-----|------|---------|---|
|       |             | Sand | Silt | TOC |      |         |   |
| R3392 | Afit #1     | 48   | 36   | 16  | Loam | 1.61    |   |
| R3393 | Afit #2     | 48   | 36   | 16  | Loam | 1.82    |   |
| R3394 | Afit #3     | 49   | 35   | 16  | Loam | 1.78    |   |

## Appendix B: Calculations of Field Capacity and Solution Concentrations for Experiments

Field capacity test of (high-clay) soil (September 18, 1998)

$M_s := 97.8 \text{ gm}$  Mass of soil in situ condition

$M_w := 18.4 \text{ gm}$  Mass of water absorbed into soil to achieve 100% FC.  
(24 hrs at saturation, 2 hrs drainage)

$$FC := \frac{M_w}{M_s} \quad FC = 0.19$$

Amount of soil with water that totals 50 grams in microcosm to achieve ~ 60% of FC of the soil

$M_{\text{soil}} := 45 \text{ gm}$  Mass of soil (in situ) to achieve ~60% of FC to equal 50 grams total mass after addition of water

$FC = 0.188$  Field capacity of water within soil to achieve 100%

$FC\% := .60$  Percentage (~60%) range of field capacity ratio determined above

$$M_{H_2O} := (M_{\text{soil}}) \cdot (FC) \cdot (FC\%) \quad M_{H_2O} = 5.1 \text{ gm}$$

$M_{H_2O} := 5.0 \text{ gm}$  <--- This is the amount of liquid added to 45 grams soil to achieve ~60% FC. Note It was rounded to 5 gm H<sub>2</sub>O to make inoculation easier within the microcosms.

$M_{sw} := 50 \text{ gm}$  <----- Mass of ~60% FC soil (Mass of soil and water together)

The addition of 5.0 grams of H<sub>2</sub>O solution (PG only, TTA only, or PG & TTA) requires a specific concentration to achieve the designed application desired in parts per million (ppm) that is equal to mg contaminant/kg soil.

### Example Calculations:

Experimental treatment of PG used in all runs ----->  $PG1000_{\text{ppm}} := 1000 \frac{\text{mg}}{\text{kg}}$

$$\text{Formula:} \quad \frac{1000 \text{ mg PG}}{1 \text{ kg soil}} = \frac{X \text{ mg PG}}{50 \text{ gm soil}}$$

Formula: 
$$X \text{ mg PG} = \frac{1000 \text{ mg PG}}{1 \text{ kg soil}} * (50 \text{ gm soil})$$

Mathcad Formula: 
$$\text{PG1000}_{\text{mass}} := (\text{PG1000}_{\text{ppm}}) \cdot (M_{\text{sw}})$$

$$\text{PG1000}_{\text{mass}} = 50 \text{ mg} \quad \text{<--- Mass of PG required for 50 grams of ~60\% FC soil} = 1,000 \text{ mg/kg}$$

Experimental treatment of TTA25 ----->  
(experimental Run-1)

$$\text{TTA}_{25\text{ppm}} := 25 \frac{\text{mg}}{\text{kg}}$$

Formula: 
$$X \text{ mg TTA} = \frac{25 \text{ mg TTA}}{1 \text{ kg soil}} * (50 \text{ gm soil})$$

Mathcad Formula: 
$$\text{TTA25}_{\text{mass}} := (\text{TTA}_{25\text{ppm}}) \cdot (M_{\text{sw}})$$

$$\text{TTA25}_{\text{mass}} = 1.25 \text{ mg} \quad \text{<-- Mass of TTA for 50 grams of ~60\% FC soil} = 25 \text{ mg/kg}$$

**Example concentration are calculated below for the solutions used in treatment of the soil (PG only, TTA only). The following formulas were used.**

Required concentration for PG1000 (50 mg PG / 50 gm soil) requires 5 mL injection into soil.

Mathcad Formula: 
$$\text{PG1000}_{\text{mass}} := (\text{PG1000}_{\text{ppm}}) \cdot (M_{\text{sw}})$$

Mathcad Formula: 
$$\text{PG1000}_{\text{conc}} := \left( \frac{\text{PG1000}_{\text{mass}}}{M_{\text{H}_2\text{O}}} \right) \cdot \left( \frac{1 \text{ gm}}{1 \text{ mL}} \right) \cdot \left( 1000 \frac{\text{mL}}{\text{L}} \right)$$

$$\text{PG1000}_{\text{conc}} = 10000 \frac{\text{mg}}{\text{L}} \quad \text{<----- Concentration required}$$

Required concentration for TTA25 (1.25 mg PG / 50 gm soil) requires 5 mL injection into soil.

Formula: 
$$\text{TTA conc} = \frac{1.25 \text{ mg TTA}}{5.0 \text{ mg H}_2\text{O}} * \frac{1 \text{ gm H}_2\text{O}}{1 \text{ mL H}_2\text{O}} * \frac{1000 \text{ mL}}{1 \text{ L}}$$

Mathcad Formula: 
$$\text{TTA25}_{\text{conc}} := \left( \frac{\text{TTA25}_{\text{mass}}}{M_{\text{H}_2\text{O}}} \right) \cdot \left( \frac{1 \text{ gm}}{1 \text{ mL}} \right) \cdot \left( 1000 \frac{\text{mL}}{\text{L}} \right)$$

$$\text{TTA25}_{\text{conc}} = 250 \frac{\text{mg}}{\text{L}} \quad \text{<----- Concentration required}$$

## Appendix C: Preparation of Solutions for Inoculation of Microcosms

### Materials used:

Chemicals used:

Propylene Glycol (aqueous), Laboratory Grade (Mallinckrodt OR, 1925: 1,2-Propanediol)

Tolyltriazole (solid), Manufacturer Grade (COBRATEC TT-100, Tolyltriazole, Sample 4239701)

Equipment used:

Flask<sub>200mL</sub> := 200 mL

Flask<sub>500mL</sub> := 500 mL

### Concentrations required for experiments:

$$\text{PG1000}_{\text{conc}} := 10000 \cdot \frac{\text{mg}}{\text{L}}$$

$$\text{TTA500}_{\text{conc}} := 5000 \cdot \frac{\text{mg}}{\text{L}}$$

$$\text{TTA25}_{\text{conc}} := 250 \cdot \frac{\text{mg}}{\text{L}}$$

$$\text{TTA750}_{\text{conc}} := 7500 \cdot \frac{\text{mg}}{\text{L}}$$

$$\text{TTA250}_{\text{conc}} := 2500 \cdot \frac{\text{mg}}{\text{L}}$$

$$\text{TTA1000}_{\text{conc}} := 10000 \cdot \frac{\text{mg}}{\text{L}}$$

### Example calculations for solution preparation of PG or TTA within a flask volume:

$$\text{Formula: } X \text{ mg material} = \text{Material conc (mg/L)} * \text{Flask volume (mL)} \cdot \frac{1 \text{ L}}{1000 \text{ mL}}$$

#### PG solution at 10,000 mg/L

$$\text{PG}_{\text{mg}} := (\text{PG1000}_{\text{conc}} \cdot \text{Flask}_{500\text{mL}}) \cdot \frac{\text{L}}{1000 \text{ mL}}$$

$$\text{PG}_{\text{mg}} = 5 \text{ } ^\circ\text{gm} \quad <-- \text{Amount of PG (liquid) mixed with 500 mL of the de-ionized water}$$

#### TTA solution at 250 mg/L

$$\text{TTA25}_{\text{mass}} := (\text{TTA25}_{\text{conc}} \cdot \text{Flask}_{200\text{mL}}) \cdot \frac{\text{L}}{1000 \text{ mL}}$$

$$\text{TTA25}_{\text{mass}} = 0.05 \text{ } ^\circ\text{gm} \quad <-- \text{Amount of TTA (solid) mixed with 200 mL of solution (de-ionized water or PG 10,000 mg/L solution)}$$

## Appendix D: Calculations for HPLC Calibration Curve for Tolyltriazole

ORIGIN=1

Known\_Concentration\_Level\_TTA :=

|      |   |
|------|---|
| 1    | The concentration of 1,000 mg/L TTA was developed first, then diluted to prepare the weaker concentrations. |
| 5    |   |
| 10   |   |
| 50   |   |
| 100  |   |
| 1000 |   |

**NOTE:** All calibration solutions are based with HPLC grade methanol. Since the extraction process of TTA from the spent soil uses a large proportion of methanol.

X := Known\_Concentration\_Level\_TTA

Table D-1  
HPLC Calibration Curve Data for Tolyltriazole

| HPLC Calibration Curve Data, Tolyltriazole |          |                     |           |                     |           |     |                           |
|--|----------|---------------------|-----------|---------------------|-----------|-----|---------------------------|
| Concentration                              |          | Run 1 (23 Sep 98)   |           | Run 2 (24 Sep 98)   |           |     |                           |
|  |          | (mAu <sup>2</sup> ) | Average   | (mAu <sup>2</sup> ) | Average   |     |                           |
| 1000 mg/L                                  | Sample 1 | 9204.9063           |           | 8981.4375           |           | --- | Run Average   Run Std Dev |
|  | Sample 2 | 9192.7627           |           | 8919.0928           |           |     |                           |
|  | Sample 3 | 9106.6846           | 9168.1179 | 8930.8477           | 8943.7927 |     |                           |
| 100 mg/L                                   | Sample 1 | 1148.9069           |           | 1120.7009           |           | --- | 1125.1359   10.4867       |
|  | Sample 2 | 1146.3660           |           | 1104.5593           |           |     |                           |
|  | Sample 3 | 1130.3009           | 1141.8579 | 1099.9812           | 1108.4138 |     |                           |
| 50 mg/L                                    | Sample 1 | 536.4797            |           | 513.9089            |           | --- | 522.2575   5.9540         |
|  | Sample 2 | 525.3796            |           | 512.1735            |           |     |                           |
|  | Sample 3 | 523.9478            | 528.6024  | 521.6556            | 515.9127  |     |                           |
| 10 mg/L                                    | Sample 1 | 112.3766            |           | 109.4433            |           | --- | 112.1975   1.4264         |
|  | Sample 2 | 115.9473            |           | 111.1758            |           |     |                           |
|  | Sample 3 | 113.1561            | 113.8267  | 111.0857            | 110.5683  |     |                           |
| 5 mg/L                                     | Sample 1 | 58.1064             |           | 56.4408             |           | --- | 57.1077   0.4723          |
|  | Sample 2 | 58.6636             |           | 56.2115             |           |     |                           |
|  | Sample 3 | 57.2977             | 58.0226   | 55.9262             | 56.1928   |     |                           |
| 1 mg/L                                     | Sample 1 | 13.1479             |           | 13.0238             |           | --- | 13.1544   0.0790          |
|  | Sample 2 | 13.3671             |           | 13.0937             |           |     |                           |
|  | Sample 3 | 13.1933             | 13.2361   | 13.1003             | 13.0726   |     |                           |

|  |           |   |
|--|-----------|---|
| Observed_Detection_Areas <sub>TTA</sub> := | 13.1544   | The detection area for each standard was performed three times and averaged to produce the data listed in "Observed_Detection_Areas <sub>TTA</sub> ".<br>$Y := \text{Observed\_Detection\_Areas}_{TTA}$ |
|  | 57.1077   |   |
|  | 112.1975  |   |
|  | 522.2575  |   |
|  | 1125.1359 |   |
|  | 9055.9553 |   |

**Calculation for the linear best fit line:**

$m := \text{slope}(X, Y)$      $m = 9.01$     <--- Calculation of slope

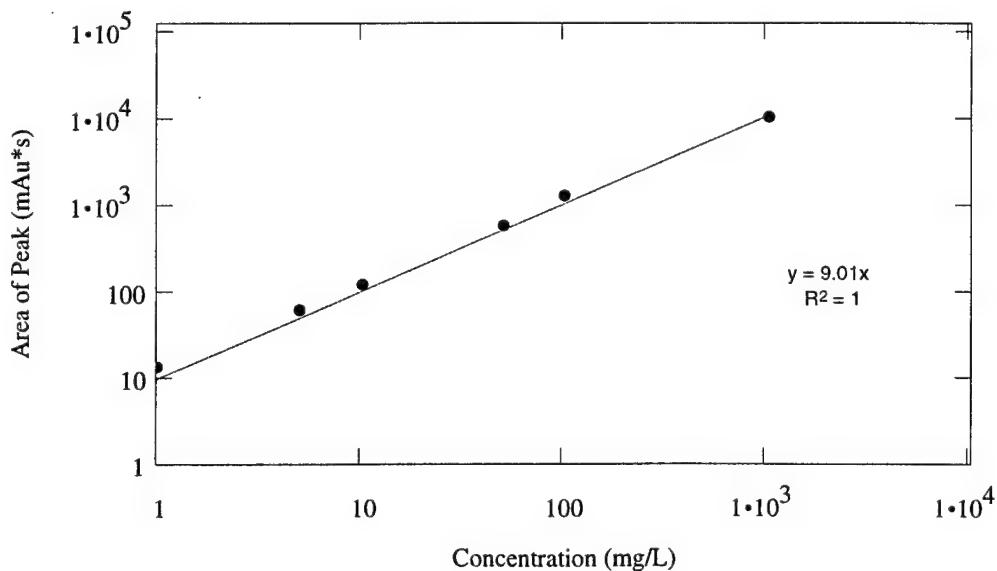
$r := \text{corr}(X, Y)$      $r = 0.9997$     <--- Calculation of the correlation between concentration and area peaks

MathCad 7.0 uses Pearson correlation coefficient

$y(x) := m \cdot x$     <--- Equation of the linear line

$y(\log x) := m \cdot \log x$     <--- Log scale is applied to enable a more usable graph, thus lower concentration levels can be calculated from the integrated areas from HPLC detection

Figure D-1  
Calibration Curve for Tolyltriazole





**Level of Detection (LOD) is provided by the formula:**

$$LOD = 3 \cdot s_{Total} \quad <--- \quad s_{Total}^2 = s_{Background}^2 + s_{Observed}^2$$

$$\sigma_{Background} := 0 \quad <--- \quad \begin{array}{l} \text{Noise is eliminated from} \\ \text{integration of areas in} \\ \text{HP Chem. Station software} \end{array}$$

$$Std\_Dev := \begin{bmatrix} 43.3375 \\ 10.4867 \\ 5.9540 \\ 1.4264 \\ .4723 \\ .0790 \end{bmatrix}$$

$$\sigma_{Observed} := \text{mean}(Std\_Dev)$$

$$\sigma_{Observed} = 10.293$$

$$\sigma_{Total} := \sqrt{\sigma_{Background}^2 + \sigma_{Observed}^2}$$

$$LOD_{areas} := 3 \cdot \sigma_{Total}$$

$$LOD_{areas} = 30.878 \quad <--- \quad \text{mAu*s}$$

$$LOD_{conc} := \frac{LOD_{areas}}{9.01}$$

$$LOD_{conc} = 3.427 \quad <--- \quad \text{mg/L}$$

## Appendix E: Respirometry Data

All respirometry experiments were conducted in accordance with the methodology section. Table E-1 is a detailed layout of all treatments for the experimental runs.

Table E-1  
Layout of All Respirometry Treatments/Experiments

|              |   |   |   |   |   |
|--------------|---|---|---|---|---|
| <b>Run 1</b> |   |   |   |   |   |
| Bottle       | 1                                       | 2                                       | 3                                       | 4                                       | 5                                       |
| Treatment    | TTA <sub>25</sub>                       | TTA <sub>25</sub>                       | TTA <sub>25</sub>                       | TTA <sub>25</sub>                       | TTA <sub>25</sub>                       |
| Soil Type    | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| Bottle       | 6                                       | 7                                       | 8                                       | 9                                       | 10                                      |
| Treatment    | Empty                                   | Empty                                   | PG <sub>1000</sub> & TTA <sub>25</sub>  | PG <sub>1000</sub> & TTA <sub>25</sub>  | PG <sub>1000</sub> & TTA <sub>25</sub>  |
| Soil Type    | Bottle                                  | Bottle                                  | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| Bottle       | 11                                      | 12                                      | 13                                      | 14                                      | 15                                      |
| Treatment    | PG <sub>1000</sub> & TTA <sub>25</sub>  | PG <sub>1000</sub> & TTA <sub>25</sub>  | Blank/H <sub>2</sub> O                  | Blank/H <sub>2</sub> O                  | Blank/H <sub>2</sub> O                  |
| Soil Type    | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| Bottle       | 16                                      | 17                                      | 18                                      | 19                                      | 20                                      |
| Treatment    | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      |
| Soil Type    | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| <b>Run 2</b> |   |   |   |   |   |
| Bottle       | 1                                       | 2                                       | 3                                       | 4                                       | 5                                       |
| Treatment    | TTA <sub>250</sub>                      | TTA <sub>250</sub>                      | TTA <sub>250</sub>                      | TTA <sub>250</sub>                      | TTA <sub>250</sub>                      |
| Soil Type    | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| Bottle       | 6                                       | 7                                       | 8                                       | 9                                       | 10                                      |
| Treatment    | Empty                                   | Empty                                   | PG <sub>1000</sub> & TTA <sub>250</sub> | PG <sub>1000</sub> & TTA <sub>250</sub> | PG <sub>1000</sub> & TTA <sub>250</sub> |
| Soil Type    | Bottle                                  | Bottle                                  | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| Bottle       | 11                                      | 12                                      | 13                                      | 14                                      | 15                                      |
| Treatment    | PG <sub>1000</sub> & TTA <sub>250</sub> | PG <sub>1000</sub> & TTA <sub>250</sub> | Blank/H <sub>2</sub> O                  | Blank/H <sub>2</sub> O                  | Blank/H <sub>2</sub> O                  |
| Soil Type    | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| Bottle       | 16                                      | 17                                      | 18                                      | 19                                      | 20                                      |
| Treatment    | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      |
| Soil Type    | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| <b>Run 3</b> |   |   |   |   |   |
| Bottle       | 1                                       | 2                                       | 3                                       | 4                                       | 5                                       |
| Treatment    | TTA <sub>500</sub>                      | TTA <sub>500</sub>                      | TTA <sub>500</sub>                      | TTA <sub>500</sub>                      | TTA <sub>500</sub>                      |
| Soil Type    | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| Bottle       | 6                                       | 7                                       | 8                                       | 9                                       | 10                                      |
| Treatment    | Empty                                   | Empty                                   | PG <sub>1000</sub> & TTA <sub>500</sub> | PG <sub>1000</sub> & TTA <sub>500</sub> | PG <sub>1000</sub> & TTA <sub>500</sub> |
| Soil Type    | Bottle                                  | Bottle                                  | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| Bottle       | 11                                      | 12                                      | 13                                      | 14                                      | 15                                      |
| Treatment    | PG <sub>1000</sub> & TTA <sub>500</sub> | PG <sub>1000</sub> & TTA <sub>500</sub> | Blank/H <sub>2</sub> O                  | Blank/H <sub>2</sub> O                  | Blank/H <sub>2</sub> O                  |
| Soil Type    | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |
| Bottle       | 16                                      | 17                                      | 18                                      | 19                                      | 20                                      |
| Treatment    | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      | PG <sub>1000</sub>                      |
| Soil Type    | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |

**Run 4**

|           |                                       |                                       |                                       |                                       |                                       |
|-----------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| Bottle    | 1                                     | 2                                     | 3                                     | 4                                     | 5                                     |
| Treatment | PG <sub>1000</sub>                    | PG <sub>1000</sub>                    | PG <sub>1000</sub>                    | PG <sub>1000</sub>                    | PG <sub>1000</sub>                    |
| Soil Type | Run-3, Bottle 1<br>TTA <sub>500</sub> | Run-3, Bottle 2<br>TTA <sub>500</sub> | Run-3, Bottle 3<br>TTA <sub>500</sub> | Run-3, Bottle 4<br>TTA <sub>500</sub> | Run-3, Bottle 5<br>TTA <sub>500</sub> |

|           |                         |                         |  |  |   |
|-----------|-------------------------|-------------------------|--|--|---|
| Bottle    | 6                       | 7                       | 8  | 9  | 10  |
| Treatment | PG <sub>1000</sub>      | PG <sub>1000</sub>      | PG <sub>1000</sub>   | PG <sub>1000</sub>   | PG <sub>1000</sub>  |
| Soil Type | Blank<br>Uncontaminated | Blank<br>Uncontaminated | Run-3, Bottle 8<br>PG <sub>1000</sub> & TTA <sub>500</sub> | Run-3, Bottle 9<br>PG <sub>1000</sub> & TTA <sub>500</sub> | Run-3, Bottle 10<br>PG <sub>1000</sub> & TTA <sub>500</sub> |

|           |   |   |                         |                         |                         |
|-----------|---|---|-------------------------|-------------------------|-------------------------|
| Bottle    | 11  | 12  | 13                      | 14                      | 15                      |
| Treatment | PG <sub>1000</sub>  | PG <sub>1000</sub>  | PG <sub>1000</sub>      | PG <sub>1000</sub>      | PG <sub>1000</sub>      |
| Soil Type | Run-3, Bottle 11<br>PG <sub>1000</sub> & TTA <sub>500</sub> | Run-3, Bottle 12<br>PG <sub>1000</sub> & TTA <sub>500</sub> | Blank<br>Uncontaminated | Blank<br>Uncontaminated | Blank<br>Uncontaminated |

|           |  |  |  |  |  |
|-----------|--|--|--|--|--|
| Bottle    | 16                                     | 17                                     | 18                                     | 19                                     | 20                                     |
| Treatment | PG <sub>1000</sub>                     | PG <sub>1000</sub>                     | PG <sub>1000</sub>                     | PG <sub>1000</sub>                     | PG <sub>1000</sub>                     |
| Soil Type | Run-3, Bottle 16<br>PG <sub>1000</sub> | Run-3, Bottle 17<br>PG <sub>1000</sub> | Run-3, Bottle 18<br>PG <sub>1000</sub> | Run-3, Bottle 19<br>PG <sub>1000</sub> | Run-3, Bottle 20<br>PG <sub>1000</sub> |

**Run 5**

|           |                    |                    |                    |                    |                    |
|-----------|--------------------|--------------------|--------------------|--------------------|--------------------|
| Bottle    | 1                  | 2                  | 3                  | 4                  | 5                  |
| Treatment | TTA <sub>750</sub> | TTA <sub>750</sub> | TTA <sub>750</sub> | TTA <sub>750</sub> | TTA <sub>750</sub> |
| Soil Type | Uncontaminated     | Uncontaminated     | Uncontaminated     | Uncontaminated     | Uncontaminated     |

|           |                    |                    |   |   |   |
|-----------|--------------------|--------------------|---|---|---|
| Bottle    | 6                  | 7                  | 8                                       | 9                                       | 10                                      |
| Treatment | PG <sub>1000</sub> | PG <sub>1000</sub> | PG <sub>1000</sub> & TTA <sub>750</sub> | PG <sub>1000</sub> & TTA <sub>750</sub> | PG <sub>1000</sub> & TTA <sub>750</sub> |
| Soil Type | Uncontaminated     | Uncontaminated     | Uncontaminated                          | Uncontaminated                          | Uncontaminated                          |

|           |   |   |                    |                    |                    |
|-----------|---|---|--------------------|--------------------|--------------------|
| Bottle    | 11                                      | 12                                      | 13                 | 14                 | 15                 |
| Treatment | PG <sub>1000</sub> & TTA <sub>750</sub> | PG <sub>1000</sub> & TTA <sub>750</sub> | PG <sub>1000</sub> | PG <sub>1000</sub> | PG <sub>1000</sub> |
| Soil Type | Uncontaminated                          | Uncontaminated                          | Uncontaminated     | Uncontaminated     | Uncontaminated     |

|           |  |  |  |  |  |
|-----------|--|--|--|--|--|
| Bottle    | 16                                       | 17                                       | 18                                       | 19                                       | 20                                       |
| Treatment | PG <sub>1000</sub> & TTA <sub>1000</sub> | PG <sub>1000</sub> & TTA <sub>1000</sub> | PG <sub>1000</sub> & TTA <sub>1000</sub> | PG <sub>1000</sub> & TTA <sub>1000</sub> | PG <sub>1000</sub> & TTA <sub>1000</sub> |
| Soil Type | Uncontaminated                           | Uncontaminated                           | Uncontaminated                           | Uncontaminated                           | Uncontaminated                           |

**Run 6**

|           |   |   |  |  |  |
|-----------|---|---|--|--|--|
| Bottle    | 1   | 2   | 3  | 4  | 5  |
| Treatment | Blank/H <sub>2</sub> O                            | Blank/H <sub>2</sub> O                            | Blank/H <sub>2</sub> O                             | Blank/H <sub>2</sub> O                             | Blank/H <sub>2</sub> O                             |
| Soil Type | Mix Run-4 & Run-5<br>Bottle 6, PG <sub>1000</sub> | Mix Run-4 & Run-5<br>Bottle 7, PG <sub>1000</sub> | Mix Run-4 & Run-5<br>Bottle 13, PG <sub>1000</sub> | Mix Run-4 & Run-5<br>Bottle 14, PG <sub>1000</sub> | Mix Run-4 & Run-5<br>Bottle 15, PG <sub>1000</sub> |

|           |                        |                        |  |  |  |
|-----------|------------------------|------------------------|--|--|--|
| Bottle    | 6                      | 7                      | 8  | 9  | 10   |
| Treatment | Blank/H <sub>2</sub> O | Blank/H <sub>2</sub> O | PG <sub>1000</sub>   | PG <sub>1000</sub>   | PG <sub>1000</sub>   |
| Soil Type | Uncontaminated<br>Soil | Uncontaminated<br>Soil | Run-5, Bottle 16<br>PG <sub>1000</sub> & TTA <sub>1000</sub> | Run-5, Bottle 17<br>PG <sub>1000</sub> & TTA <sub>1000</sub> | Run-5, Bottle 18<br>PG <sub>1000</sub> & TTA <sub>1000</sub> |

|           |  |  |                        |                        |                        |
|-----------|--|--|------------------------|------------------------|------------------------|
| Bottle    | 11   | 12   | 13                     | 14                     | 15                     |
| Treatment | PG <sub>1000</sub>   | PG <sub>1000</sub>   | Blank/H <sub>2</sub> O | Blank/H <sub>2</sub> O | Blank/H <sub>2</sub> O |
| Soil Type | Run-5, Bottle 19<br>PG <sub>1000</sub> & TTA <sub>1000</sub> | Run-5, Bottle 20<br>PG <sub>1000</sub> & TTA <sub>1000</sub> | Uncontaminated<br>Soil | Uncontaminated<br>Soil | Uncontaminated<br>Soil |

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Figure E-1 Averaged Cumulative O<sub>2</sub> Consumption (uL), Experimental Run-1

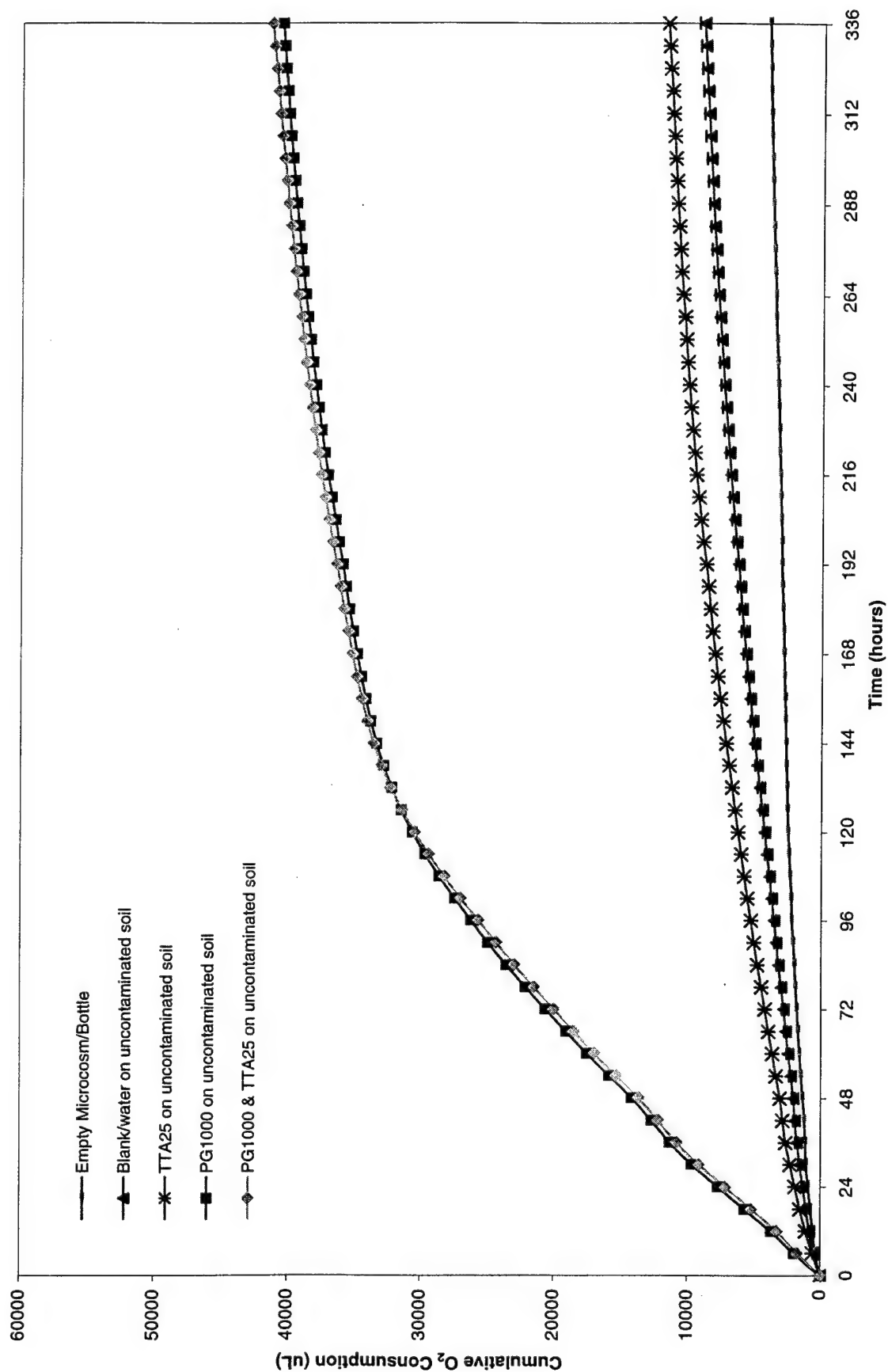


Figure E-2 Averaged Cumulative CO<sub>2</sub> Production (uL), Experimental Run-1

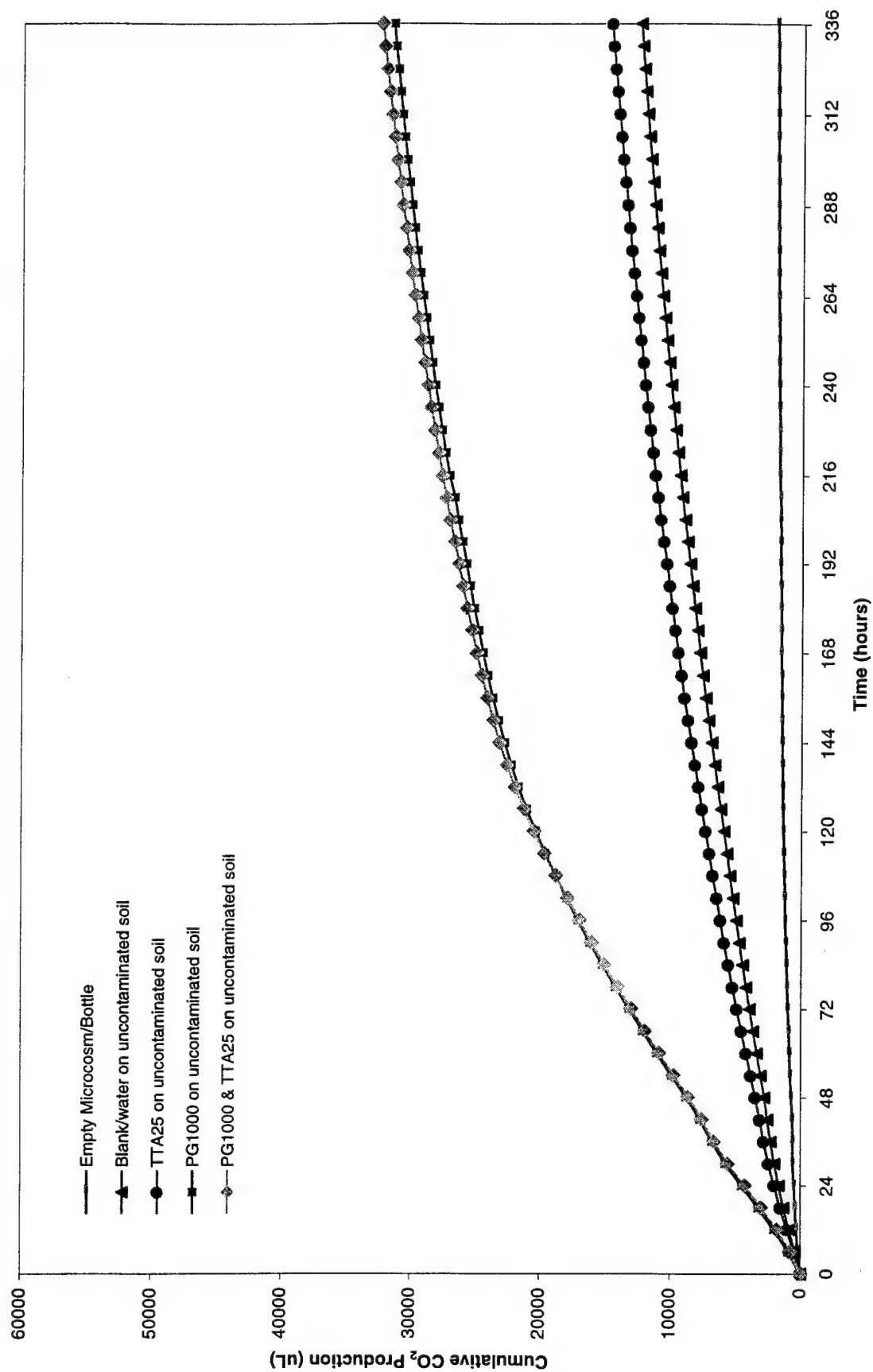


Figure E-3 Averaged  $O_2/CO_2$  Ratio (uL/uL), Experimental Run-1

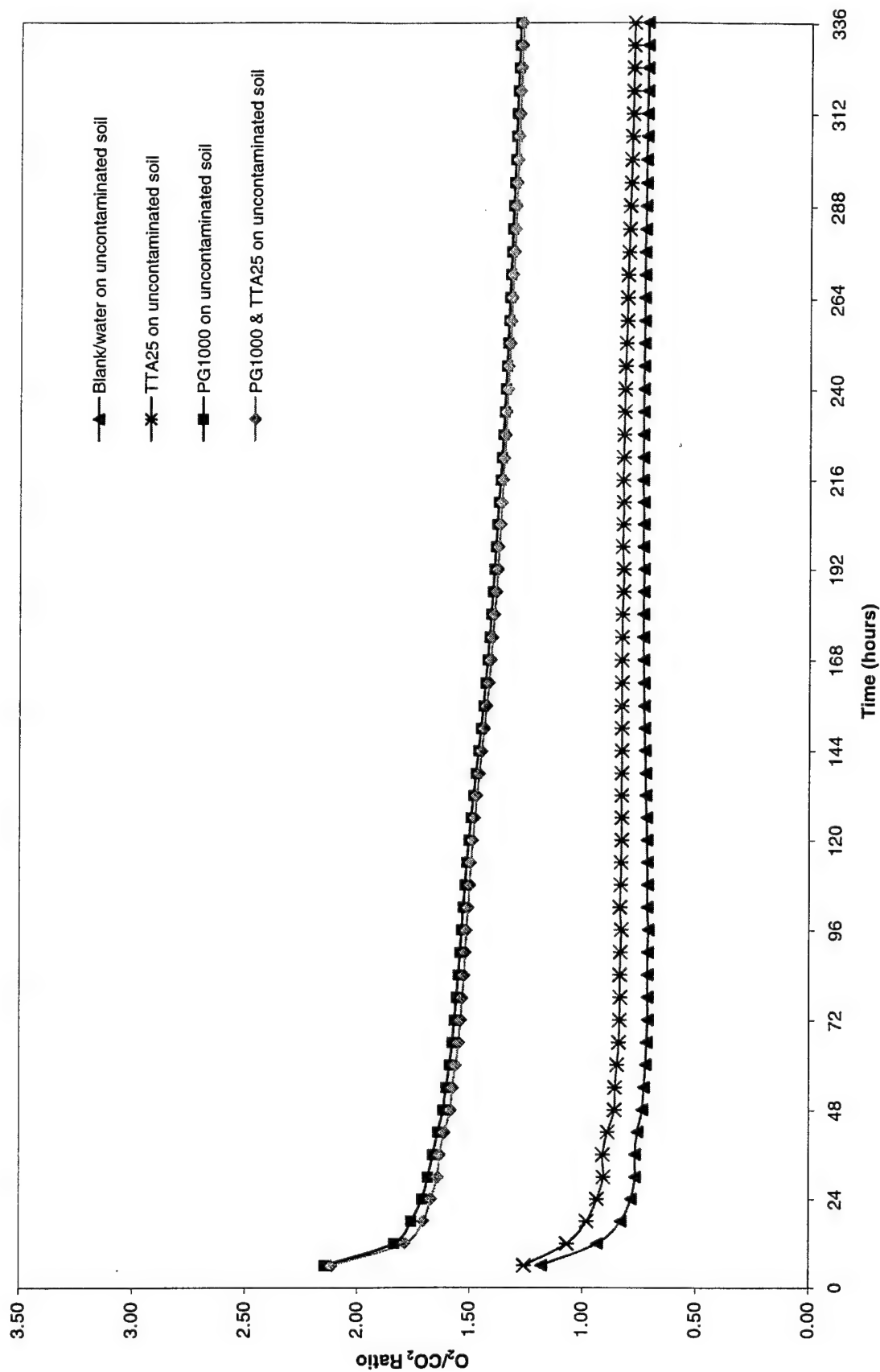


Figure E-4 Averaged Rate of O<sub>2</sub> Consumption (uL/hr), Experimental Run-1

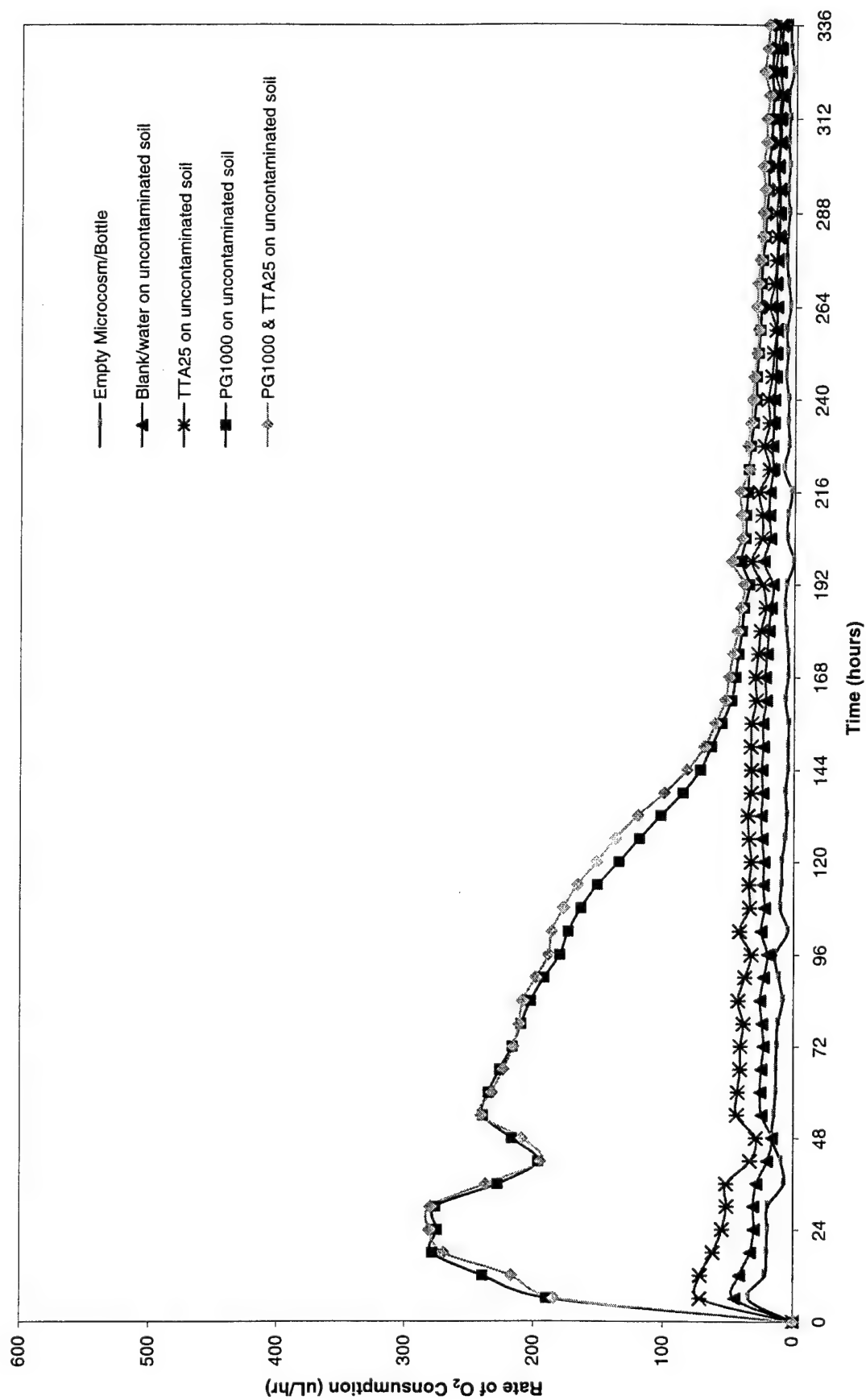




Figure E-5 Averaged Rate of CO<sub>2</sub> Production (uL/hr), Experimental Run-1

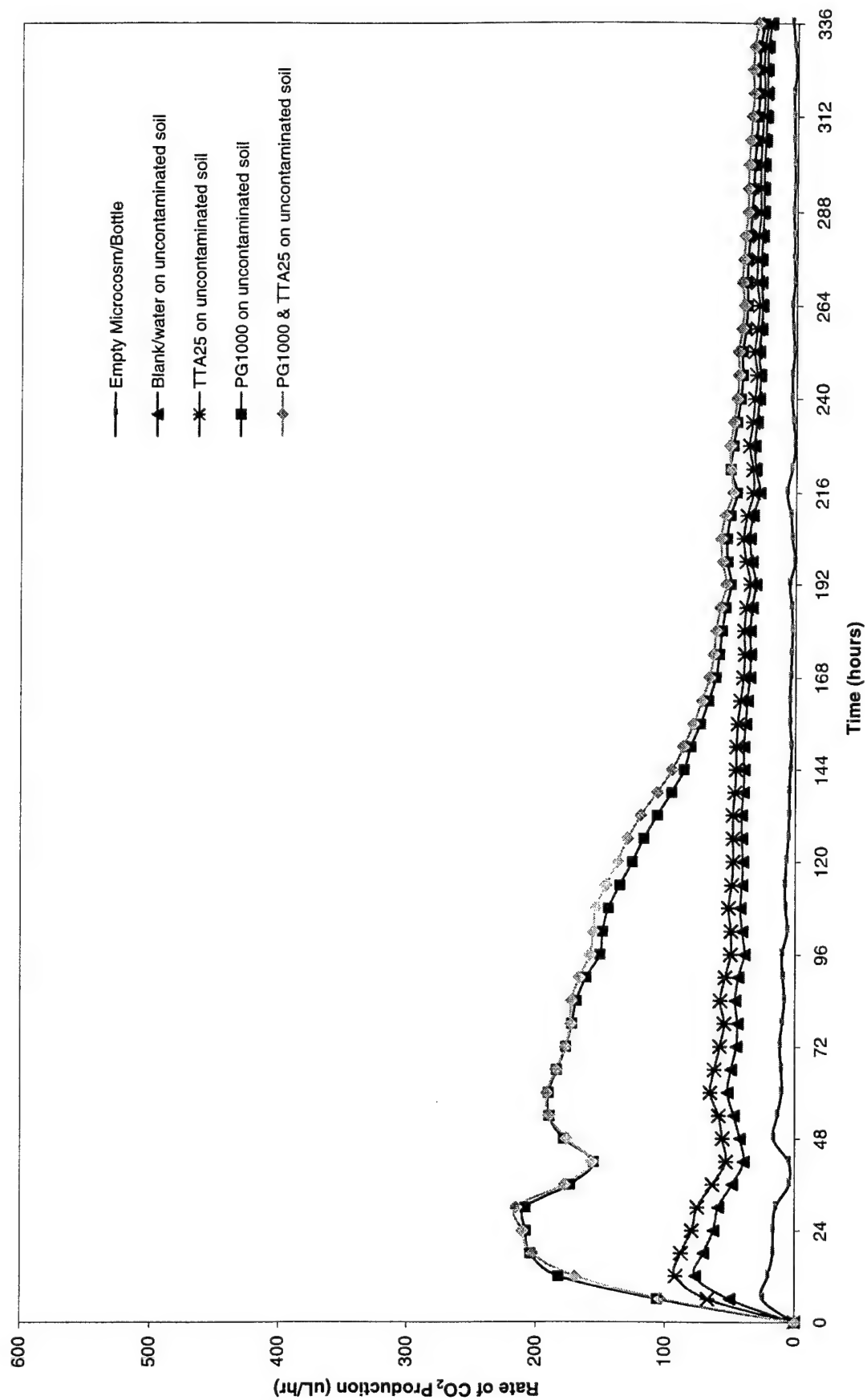


Figure E-6 Averaged Cumulative O<sub>2</sub> Consumption (uL), Experimental Run-2

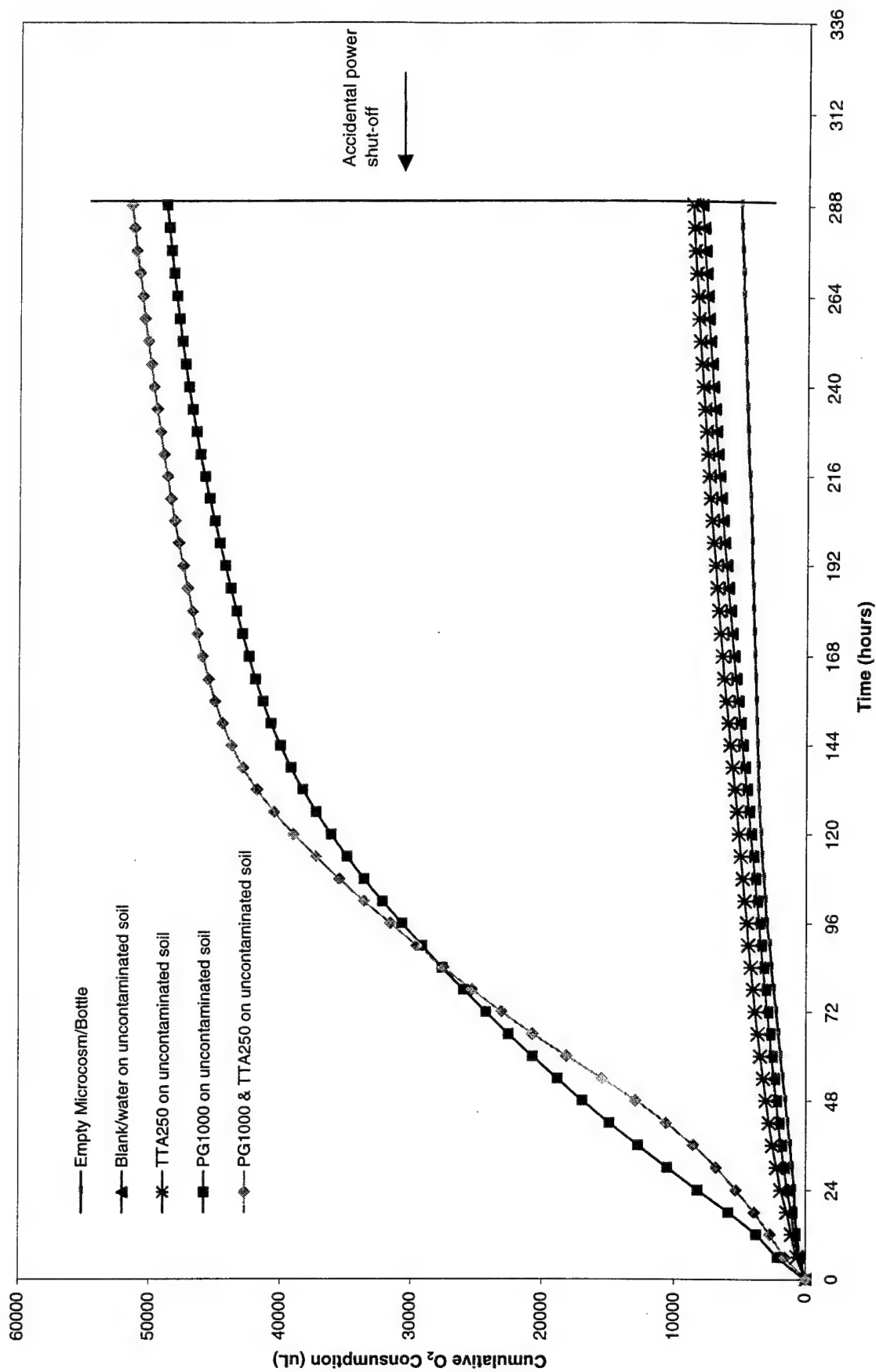


Figure E-7 Averaged Rate of O<sub>2</sub> Consumption (uL/hr), Experimental Run-2

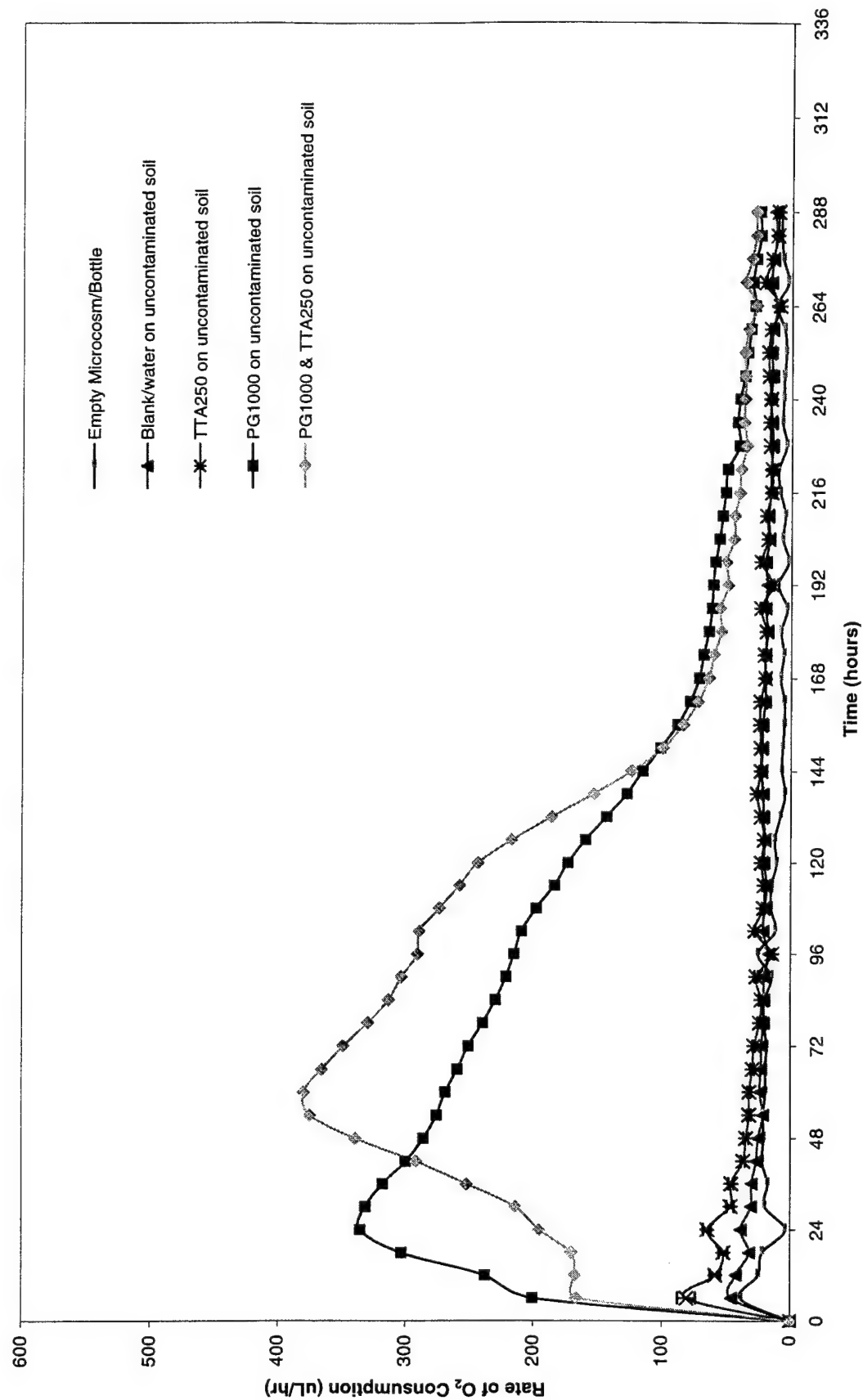


Figure E-8 Averaged Cumulative O<sub>2</sub> Consumption (uL), Experimental Run-2 (Re-accomplished)

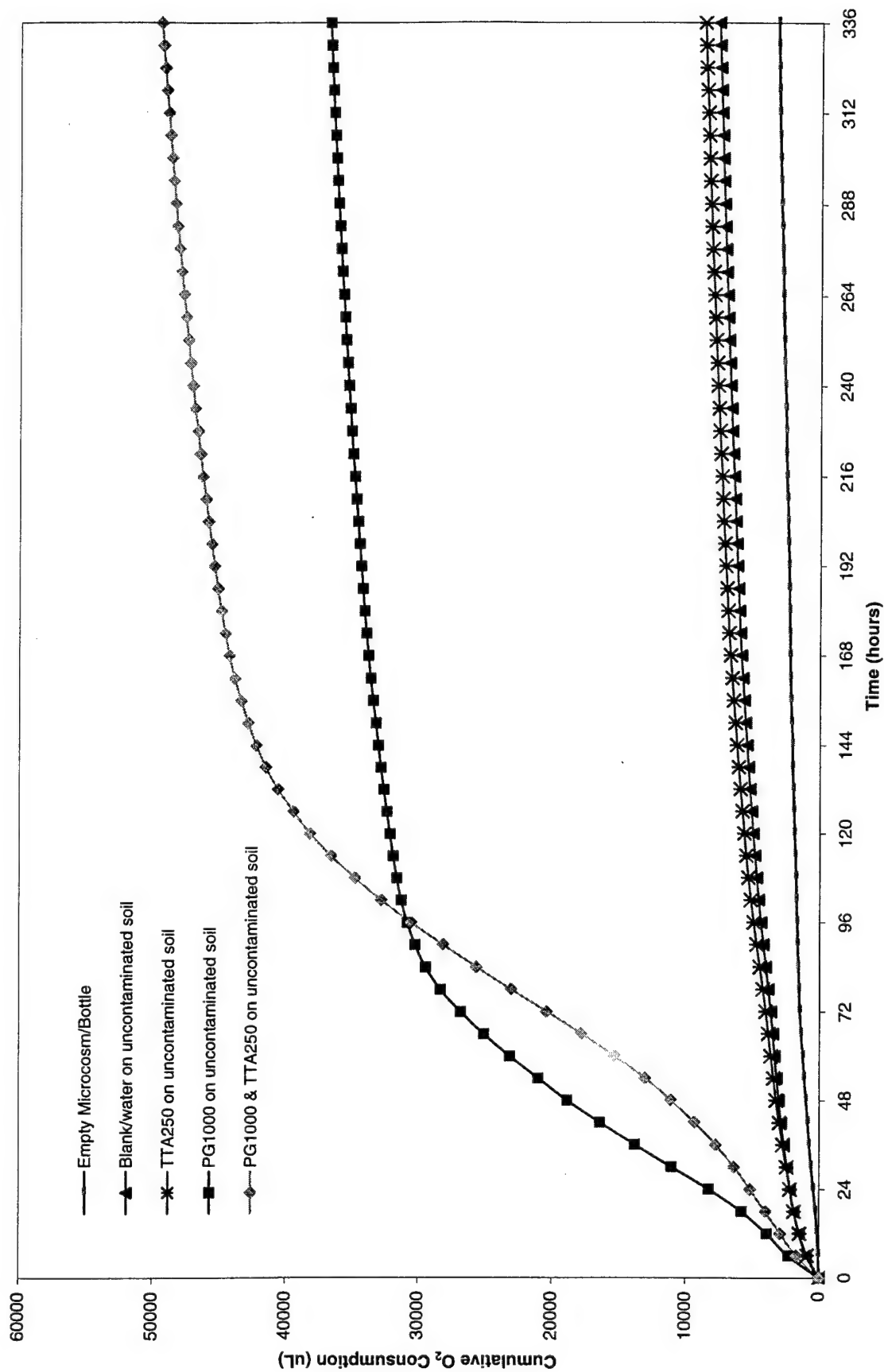


Figure E-9 Averaged Rate of O<sub>2</sub> Consumption (uL/hr), Experimental Run-2 (Re-accomplished)

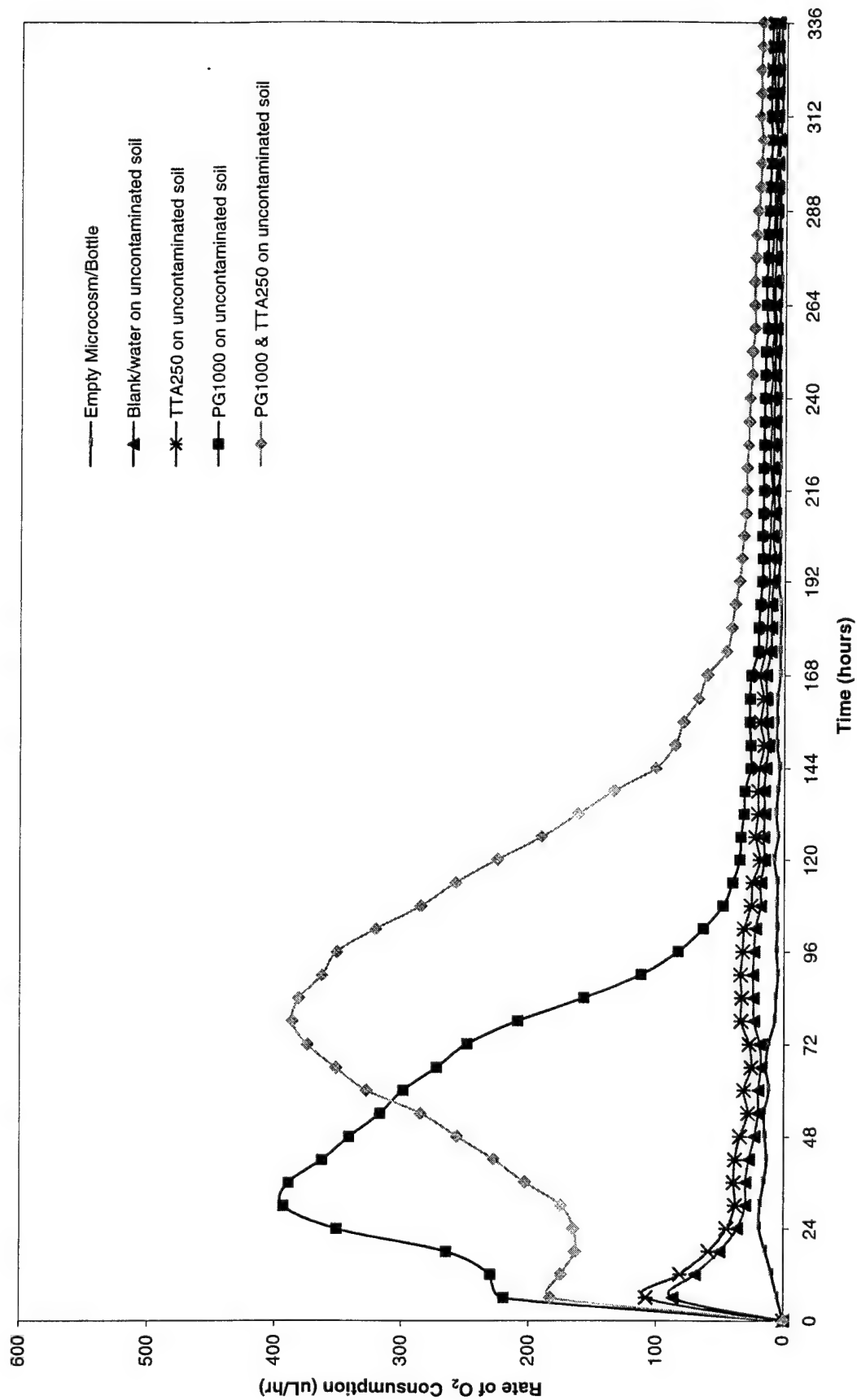


Figure E-10 Averaged Cumulative O<sub>2</sub> Consumption (uL), Experimental Run-3

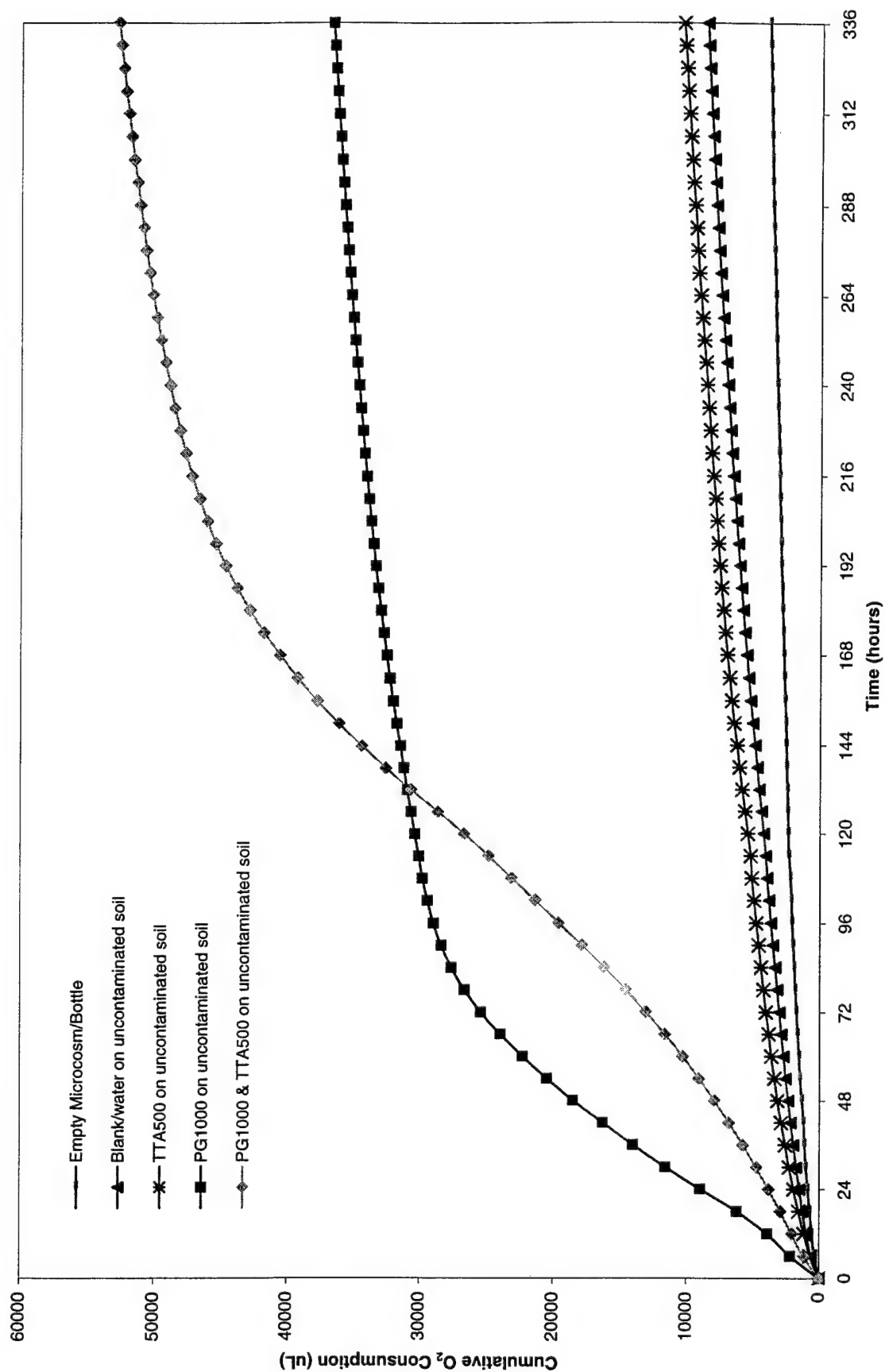


Figure E-11 Averaged Rate of O<sub>2</sub> Consumption (uL/hr), Experimental Run-3

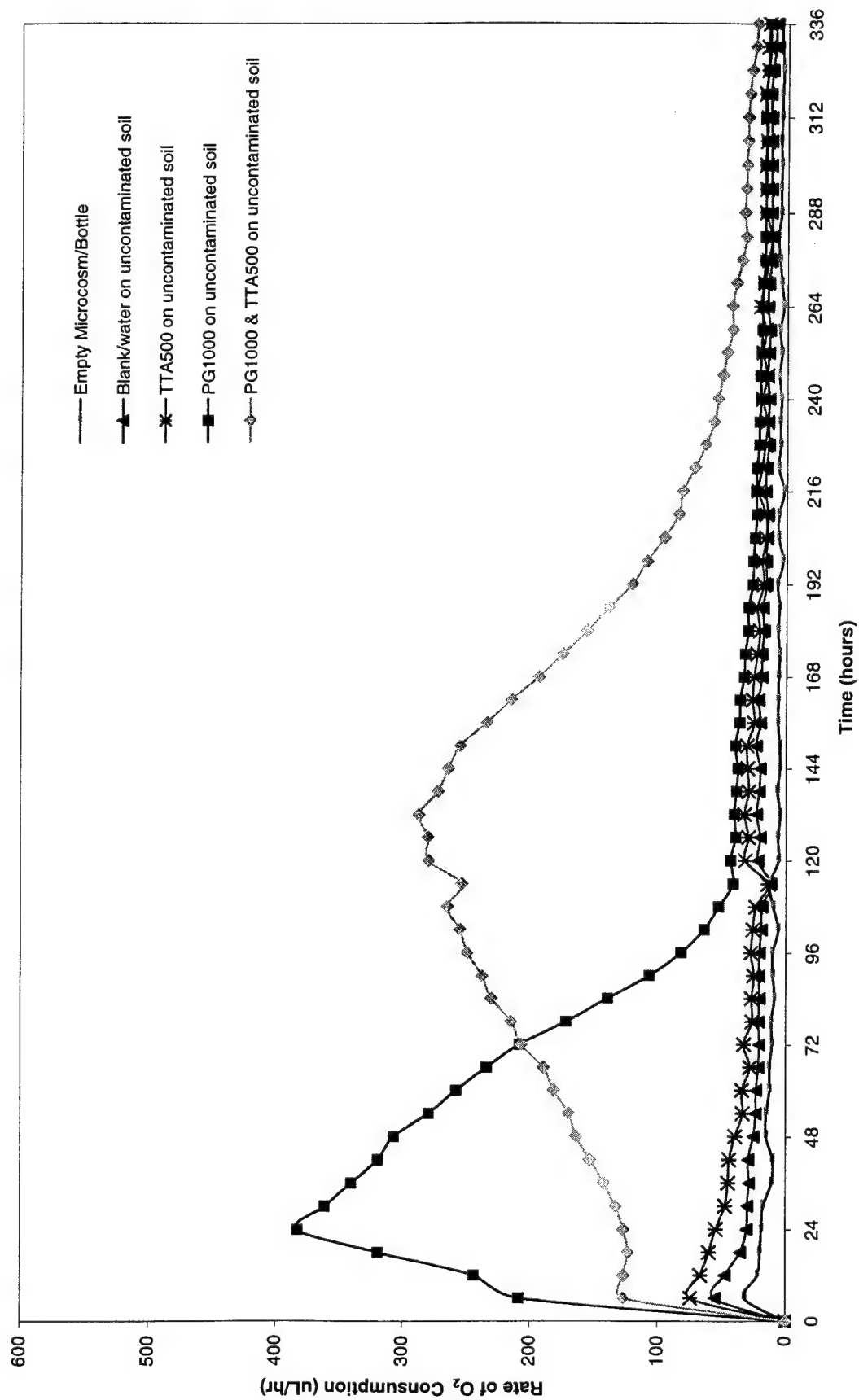


Figure E-12 Averaged Cumulative O<sub>2</sub> Consumption (uL), Experimental Run-4

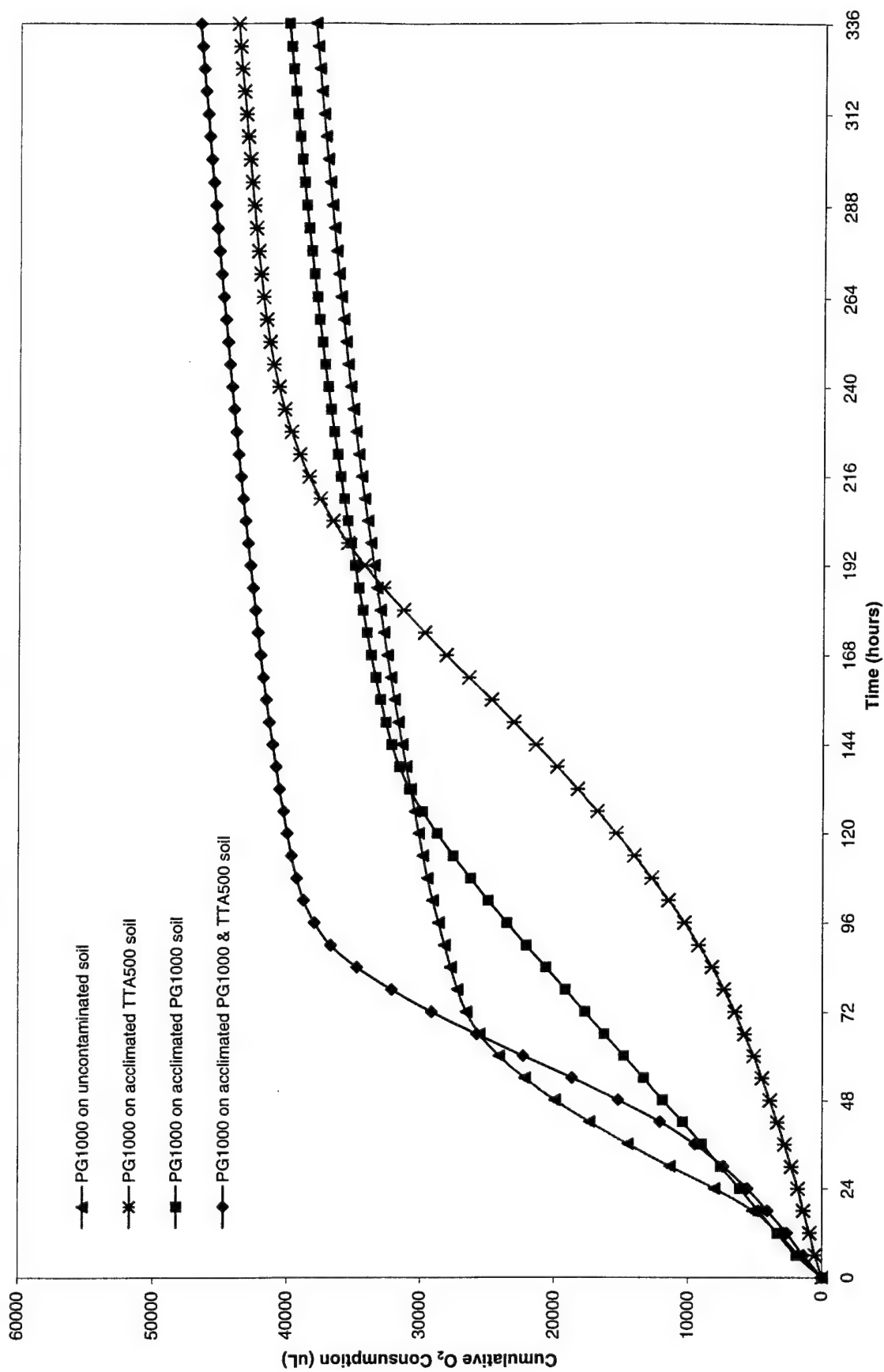




Figure E-13 Averaged Rate of O<sub>2</sub> Consumption (uL/hr), Experimental Run-4

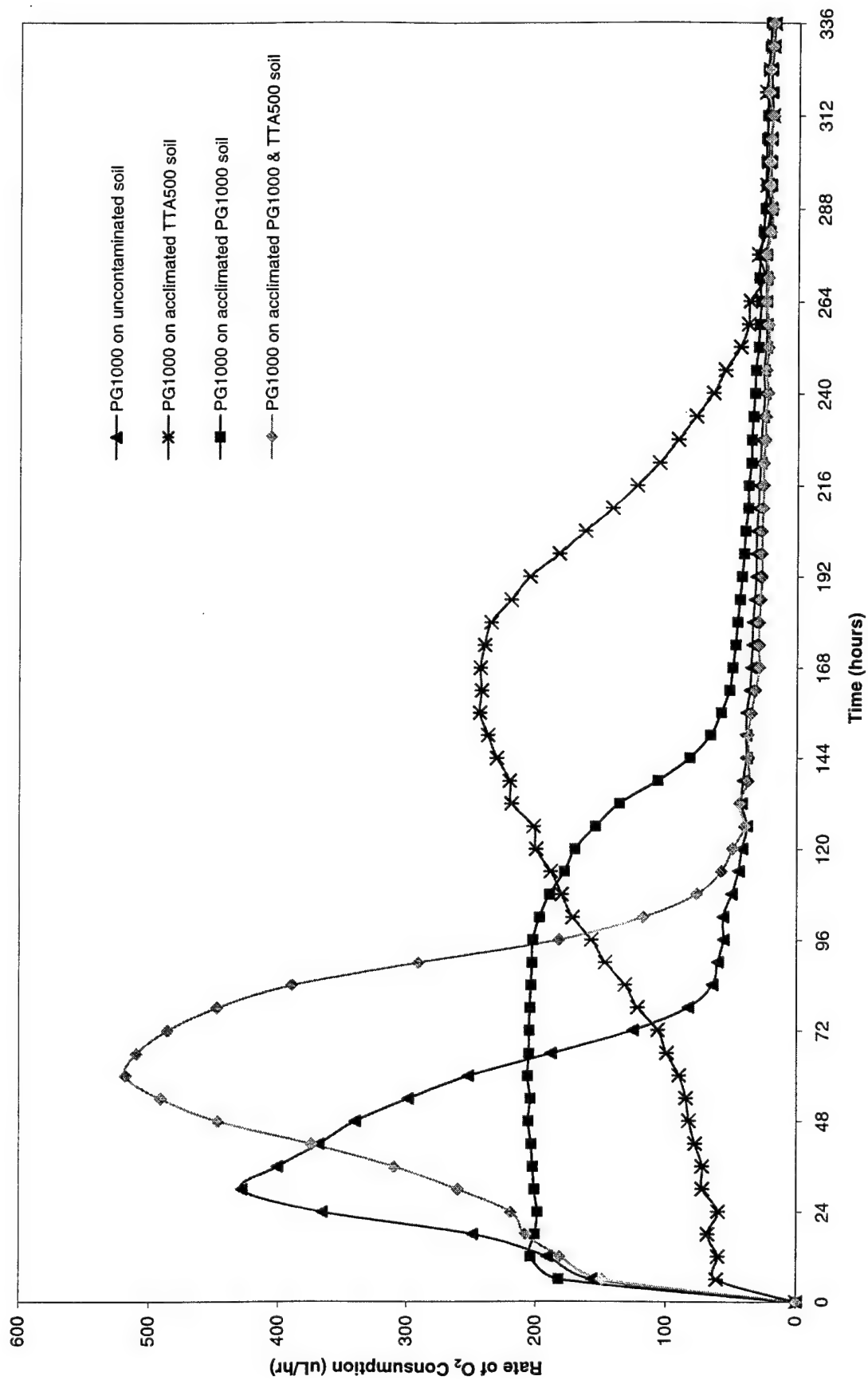


Figure E-14 Averaged Cumulative O<sub>2</sub> Consumption (uL), Experimental Run-5

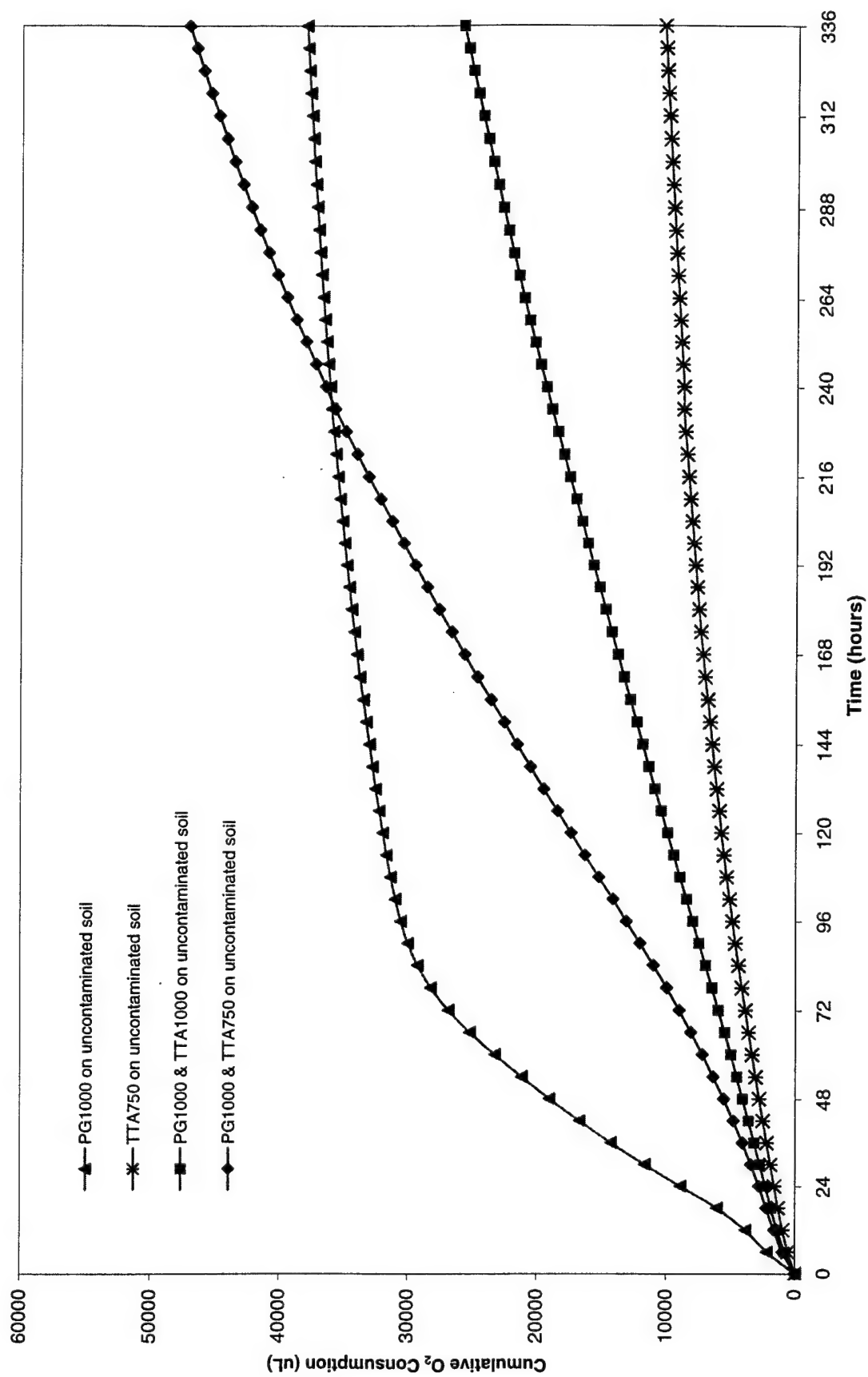


Figure E-15 Averaged Rate of O<sub>2</sub> Consumption (uL/hr), Experimental Run-5

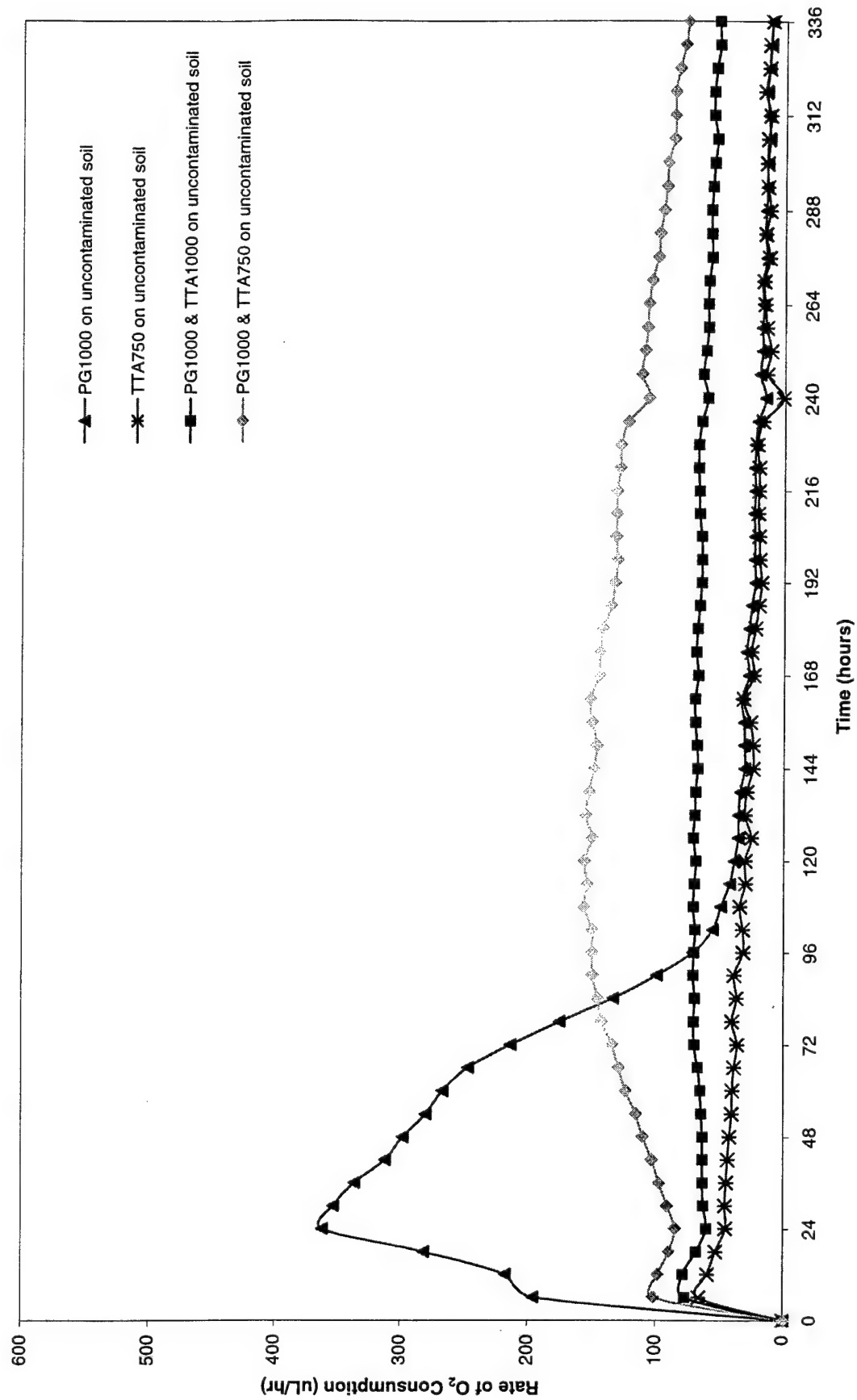


Figure E-16 Averaged Cumulative O<sub>2</sub> Consumption (uL), Experimental Run-6

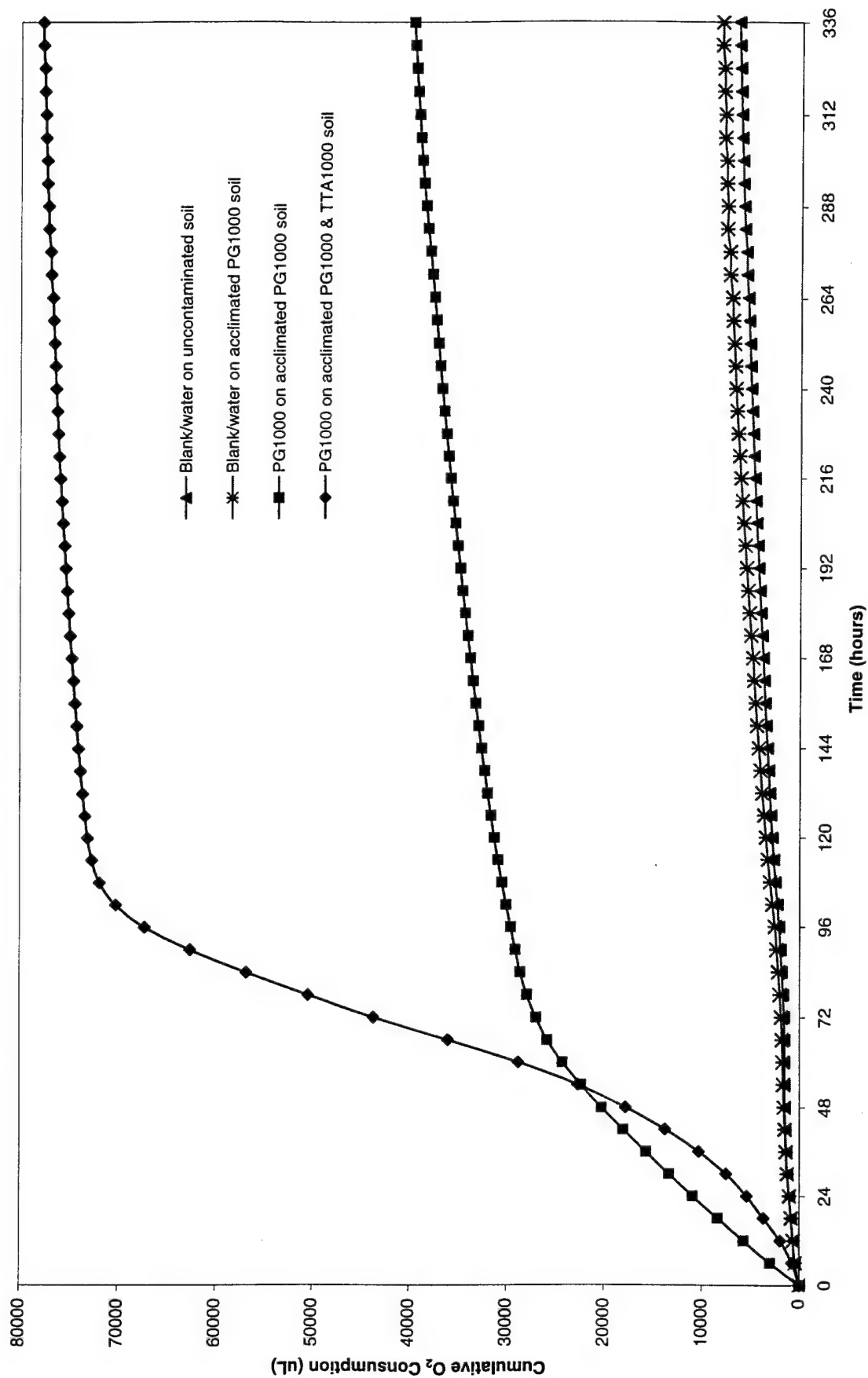
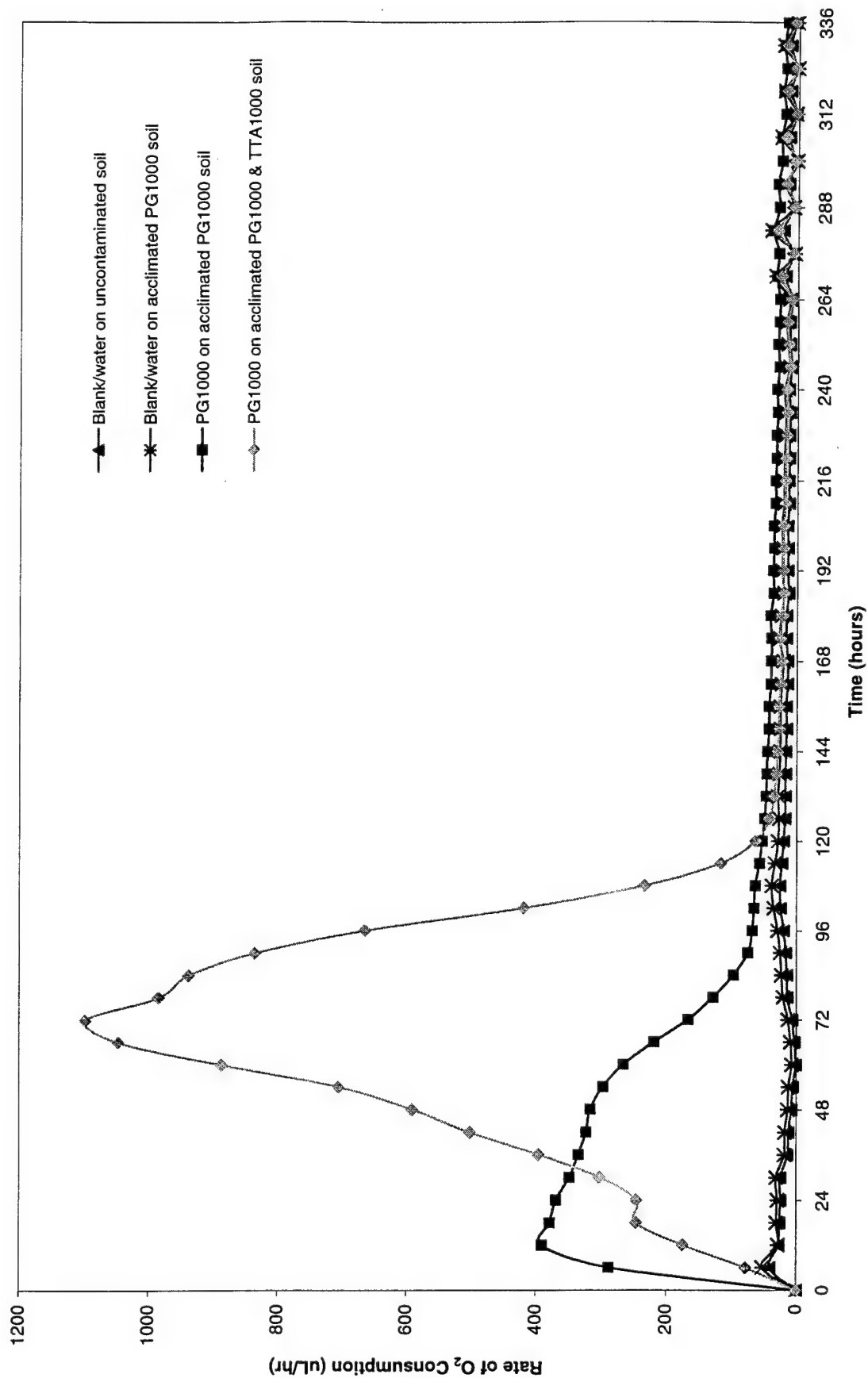


Figure E-17 Averaged Rate of O<sub>2</sub> Consumption (uL/hr), Experimental Run-6



## **Appendix F: Statistical Procedures for Determining Biodegradation Effects from the Addition of Individual ADF Chemicals (Propylene Glycol or Tolyltriazole) on Uncontaminated Soil**

The data listed in the following five tables and figures explains the possible interaction (decreased/no influence/increased) of biodegradation from individual chemical components (PG or TTA) upon a soil environment. This determination was made using the O<sub>2</sub> consumption totals of the contaminated soil with (PG or TTA) against the uncontaminated soil. A two-sample t-test was performed using a significance level of  $\alpha = 0.05$ . A 95% CI was developed from the t-test results to depict the O<sub>2</sub> consumption effects. Both populations were assumed normal and the two population variances were assumed equal.

H<sub>0</sub>: There was no effect on the O<sub>2</sub> consumption due to the contaminant addition  
H<sub>a</sub>: There was an effect (decreased or increased) on the O<sub>2</sub> consumption due to the contaminant addition

The pooled estimator, which is an estimate of the common population variance was determined by using the following equation (Devore, 358):

$$S_p^2 = \frac{(n_1-1)*S_1^2 + (n_2-1)*S_2^2}{(n_1+n_2) - 2}$$

Where  $n_1$  and  $n_2$  are the sample sizes of the respective treatments, and  $S_1$  and  $S_2$  are the standard deviations of the respective treatments.

The standard error was determined by the following equations (Devore, 358):

$$\text{Std-Error} = S_p (1/n_1 + 1/n_2)^{1/2}$$

The calculated t-statistic (t) was then determined by dividing the difference of the means by the standard error.

$$t = \frac{(X_{\text{chemical}} - X_{\text{soil}})}{(\text{Std-Error})}$$

The t-critical ( $t_{crit}$ ) was determined for a two-tailed t-test since the effects on biodegradation may be enhanced or inhibited as the alternate hypothesis, thus  $\alpha/2$  was used.

$$t_{crit} = t_{\alpha/2, n_1+n_2-2} = 2.447 \text{ (Devore, 707)}$$

Given:  $\alpha = 0.05$  (95% confidence interval)

$n_1 = 3$  (number of blank microcosms)

$n_2 = 5$  (number of chemical microcosms)

The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical.

The t-critical ( $t_{crit}$ ) was determined for a two-tailed test since the effects on biodegradation may be enhanced or inhibited as the alternate hypothesis. The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical. An example of the test statistic is shown below:

|                    |                 |                |
|--------------------|-----------------|----------------|
| $t \leq -t_{crit}$ | $t \leq -2.447$ | Inhibition     |
| $t \geq t_{crit}$  | $t \geq 2.447$  | Biodegradation |

The upper and lower 95% CI were determined by using the following equation (Devore, 361). This data was shown with the difference of the means (for the sample at its particular position on the time line) in Figures F-1 through F-5.

$$\text{Equation Format: } (X_{\text{chemical}} - X_{\text{soil}}) \pm (t_{\alpha/2, n_1+n_2-2}) * (S_p) * (1/n_1 + 1/n_2)^{1/2}$$

$X_{\text{soil}}$  = Uncontaminated soil is the control

$X_{\text{chemical}}$  = PG only -or- TTA only concentration amount

All observation points (every 6 hours) were statically tested for the entire respirometry period of 2 weeks.

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Table F-1 (Run-1) Data ( $O_2$ ) for Determining Biodegradation from the Individual Treatment of 25 mg/kg Tolytriazole on Uncontaminated Soil

| Time (hours) | Mean Sol ( $\mu$ L) | Std Dev Sol | Mean TTA25 in Sol ( $\mu$ L) | Std Dev TTA25 in Sol | Pooled Estimator $S_p^2$ | Standard Error | $X_{TTA25} - X_{coll}$ | Calc T Value ( $T_{crit} = 2.447$ ) | Upper 95% CI | Lower 95% CI | Biodegradation /inhibition/ No effect |
|--------------|---------------------|-------------|------------------------------|----------------------|--------------------------|----------------|------------------------|-------------------------------------|--------------|--------------|---------------------------------------|
| 0            | 0                   | 0           | 0                            | 0                    | 0                        | 0              | 0                      | 0.000                               | 0            | 0            | NA                                    |
| 6            | 495                 | 56          | 603                          | 104                  | 8233                     | 66             | 108                    | 1.628                               | 270          | -54          | No Effect                             |
| 12           | 818                 | 72          | 1100                         | 270                  | 50445                    | 164            | 282                    | 1.720                               | 683          | -119         | No Effect                             |
| 18           | 1075                | 79          | 1529                         | 422                  | 120783                   | 254            | 454                    | 1.788                               | 1075         | -167         | No Effect                             |
| 24           | 1309                | 82          | 1908                         | 569                  | 218415                   | 341            | 599                    | 1.754                               | 1434         | -236         | No Effect                             |
| 30           | 1548                | 90          | 2263                         | 730                  | 357732                   | 437            | 715                    | 1.637                               | 1784         | -354         | No Effect                             |
| 36           | 1789                | 101         | 2620                         | 876                  | 515345                   | 524            | 851                    | 1.624                               | 2134         | -432         | No Effect                             |
| 42           | 1926                | 112         | 2847                         | 1002                 | 673928                   | 600            | 922                    | 1.537                               | 2389         | -545         | No Effect                             |
| 48           | 2049                | 113         | 3047                         | 1138                 | 868196                   | 680            | 999                    | 1.468                               | 2664         | -666         | No Effect                             |
| 54           | 2240                | 114         | 3349                         | 1281                 | 1097665                  | 765            | 1109                   | 1.449                               | 2981         | -763         | No Effect                             |
| 60           | 2431                | 112         | 3645                         | 1417                 | 1342338                  | 846            | 1214                   | 1.434                               | 3284         | -857         | No Effect                             |
| 66           | 2619                | 113         | 3929                         | 1556                 | 1619299                  | 929            | 1310                   | 1.410                               | 3584         | -964         | No Effect                             |
| 72           | 2797                | 119         | 4212                         | 1690                 | 1909109                  | 1009           | 1415                   | 1.402                               | 3884         | -1054        | No Effect                             |
| 78           | 2986                | 126         | 4477                         | 1819                 | 2210960                  | 1086           | 1492                   | 1.374                               | 4149         | -1166        | No Effect                             |
| 84           | 3186                | 132         | 4774                         | 1934                 | 2490896                  | 1155           | 1587                   | 1.375                               | 4413         | -1238        | No Effect                             |
| 90           | 3362                | 138         | 5033                         | 2040                 | 2782002                  | 1218           | 1671                   | 1.372                               | 4651         | -1310        | No Effect                             |
| 96           | 3518                | 143         | 5259                         | 2136                 | 3049086                  | 1275           | 1740                   | 1.365                               | 4861         | -1380        | No Effect                             |
| 102          | 3709                | 147         | 5547                         | 2226                 | 3310132                  | 1329           | 1838                   | 1.383                               | 5089         | -1414        | No Effect                             |
| 108          | 3880                | 151         | 5779                         | 2313                 | 3573859                  | 1381           | 1899                   | 1.375                               | 5277         | -1480        | No Effect                             |
| 114          | 4058                | 157         | 6015                         | 2396                 | 3836501                  | 1430           | 1957                   | 1.368                               | 5457         | -1543        | No Effect                             |
| 120          | 4238                | 162         | 6239                         | 2476                 | 4094449                  | 1478           | 2002                   | 1.354                               | 5618         | -1614        | No Effect                             |
| 126          | 4428                | 168         | 6477                         | 2545                 | 4327367                  | 1519           | 2049                   | 1.349                               | 5767         | -1668        | No Effect                             |
| 132          | 4622                | 172         | 6719                         | 2611                 | 4554081                  | 1558           | 2096                   | 1.345                               | 5910         | -1717        | No Effect                             |
| 138          | 4808                | 176         | 6940                         | 2673                 | 4774770                  | 1596           | 2132                   | 1.336                               | 6037         | -1773        | No Effect                             |
| 144          | 4996                | 182         | 7165                         | 2730                 | 4981328                  | 1630           | 2169                   | 1.331                               | 6158         | -1819        | No Effect                             |
| 150          | 5181                | 183         | 7389                         | 2783                 | 5175384                  | 1661           | 2209                   | 1.329                               | 6274         | -1857        | No Effect                             |
| 156          | 5366                | 188         | 7612                         | 2837                 | 5377402                  | 1694           | 2246                   | 1.326                               | 6390         | -1898        | No Effect                             |
| 162          | 5534                | 189         | 7808                         | 2882                 | 5549173                  | 1720           | 2274                   | 1.322                               | 6484         | -1935        | No Effect                             |
| 168          | 5705                | 196         | 8013                         | 2925                 | 5718301                  | 1746           | 2308                   | 1.321                               | 6581         | -1966        | No Effect                             |
| 174          | 5862                | 203         | 8204                         | 2966                 | 5880093                  | 1771           | 2342                   | 1.322                               | 6675         | -1991        | No Effect                             |
| 180          | 6015                | 206         | 8379                         | 3009                 | 6051805                  | 1797           | 2364                   | 1.316                               | 6760         | -2032        | No Effect                             |
| 186          | 6151                | 215         | 8531                         | 3048                 | 6207598                  | 1820           | 2380                   | 1.308                               | 6833         | -2072        | No Effect                             |
| 192          | 6277                | 219         | 8700                         | 3083                 | 6352236                  | 1841           | 2423                   | 1.316                               | 6927         | -2081        | No Effect                             |
| 198          | 6452                | 233         | 8928                         | 3119                 | 6501484                  | 1862           | 2476                   | 1.330                               | 7033         | -2081        | No Effect                             |
| 204          | 6595                | 237         | 9103                         | 3153                 | 6647500                  | 1883           | 2507                   | 1.332                               | 7115         | -2100        | No Effect                             |
| 210          | 6743                | 247         | 9271                         | 3189                 | 6801504                  | 1905           | 2528                   | 1.327                               | 7189         | -2132        | No Effect                             |
| 216          | 6888                | 259         | 9461                         | 3222                 | 6945322                  | 1925           | 2573                   | 1.337                               | 7283         | -2136        | No Effect                             |
| 222          | 7016                | 264         | 9595                         | 3256                 | 7091455                  | 1945           | 2579                   | 1.326                               | 7338         | -2180        | No Effect                             |
| 228          | 7149                | 267         | 9755                         | 3288                 | 7231037                  | 1964           | 2606                   | 1.327                               | 7411         | -2200        | No Effect                             |
| 234          | 7278                | 268         | 9891                         | 3314                 | 7346271                  | 1979           | 2613                   | 1.320                               | 7457         | -2230        | No Effect                             |
| 240          | 7404                | 272         | 10034                        | 3341                 | 7467582                  | 1996           | 2629                   | 1.317                               | 7513         | -2254        | No Effect                             |
| 246          | 7517                | 276         | 10157                        | 3369                 | 7594325                  | 2013           | 2640                   | 1.312                               | 7565         | -2285        | No Effect                             |
| 252          | 7627                | 282         | 10271                        | 3398                 | 7723754                  | 2030           | 2645                   | 1.303                               | 7611         | -2322        | No Effect                             |
| 258          | 7739                | 286         | 10374                        | 3424                 | 7842578                  | 2045           | 2653                   | 1.288                               | 7639         | -2370        | No Effect                             |
| 264          | 7849                | 288         | 10508                        | 3447                 | 7947922                  | 2059           | 2659                   | 1.292                               | 7698         | -2379        | No Effect                             |

Table F-1 (Run-1) Data (O<sub>2</sub>) for Determining Biodegradation from the Individual Treatment of 25 mg/kg Tolytriazole on Uncontaminated Soil

| Time (hours) | Mean Soil (uL) | Std Dev Soil | Mean TTA25 in Soil (uL) | Std Dev TTA25 in Soil | Pooled Estimator S <sub>p</sub> <sup>2</sup> | Standard Error | X <sub>TTA25</sub> - X <sub>sol</sub> | Calc T Value (T <sub>crit</sub> = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /inhibition/ No effect |
|--------------|----------------|--------------|-------------------------|-----------------------|--|----------------|---------------------------------------|--|--------------|--------------|---------------------------------------|
| 270          | 7962           | 297          | 10619                   | 3475                  | 8077715                                      | 2076           | 2657                                  | 1.280                                    | 7736         | -2422        | No Effect                             |
| 276          | 8066           | 308          | 10721                   | 3500                  | 8196647                                      | 2091           | 2655                                  | 1.270                                    | 7771         | -2461        | No Effect                             |
| 282          | 8166           | 314          | 10819                   | 3527                  | 8324805                                      | 2107           | 2653                                  | 1.259                                    | 7809         | -2503        | No Effect                             |
| 288          | 8264           | 320          | 10922                   | 3551                  | 8441319                                      | 2122           | 2658                                  | 1.253                                    | 7850         | -2534        | No Effect                             |
| 294          | 8359           | 331          | 11016                   | 3575                  | 8555635                                      | 2136           | 2657                                  | 1.244                                    | 7885         | -2570        | No Effect                             |
| 300          | 8462           | 337          | 11117                   | 3598                  | 8670291                                      | 2150           | 2655                                  | 1.235                                    | 7917         | -2607        | No Effect                             |
| 306          | 8555           | 343          | 11207                   | 3622                  | 8786387                                      | 2165           | 2653                                  | 1.225                                    | 7950         | -2644        | No Effect                             |
| 312          | 8643           | 355          | 11302                   | 3645                  | 8897176                                      | 2178           | 2659                                  | 1.221                                    | 7989         | -2672        | No Effect                             |
| 318          | 8727           | 360          | 11386                   | 3669                  | 9016700                                      | 2193           | 2659                                  | 1.213                                    | 8025         | -2707        | No Effect                             |
| 324          | 8820           | 370          | 11501                   | 3691                  | 9127716                                      | 2206           | 2682                                  | 1.215                                    | 8081         | -2717        | No Effect                             |
| 330          | 8910           | 373          | 11598                   | 3712                  | 9230573                                      | 2219           | 2688                                  | 1.212                                    | 8118         | -2741        | No Effect                             |
| 336          | 8992           | 373          | 11684                   | 3732                  | 9329889                                      | 2231           | 2692                                  | 1.207                                    | 8151         | -2766        | No Effect                             |

**Figure F-1 Difference Between the Means ( $O_2$ ) and 95% CI for  
25 mg/kg Tolyltriazole on Uncontaminated Soil**

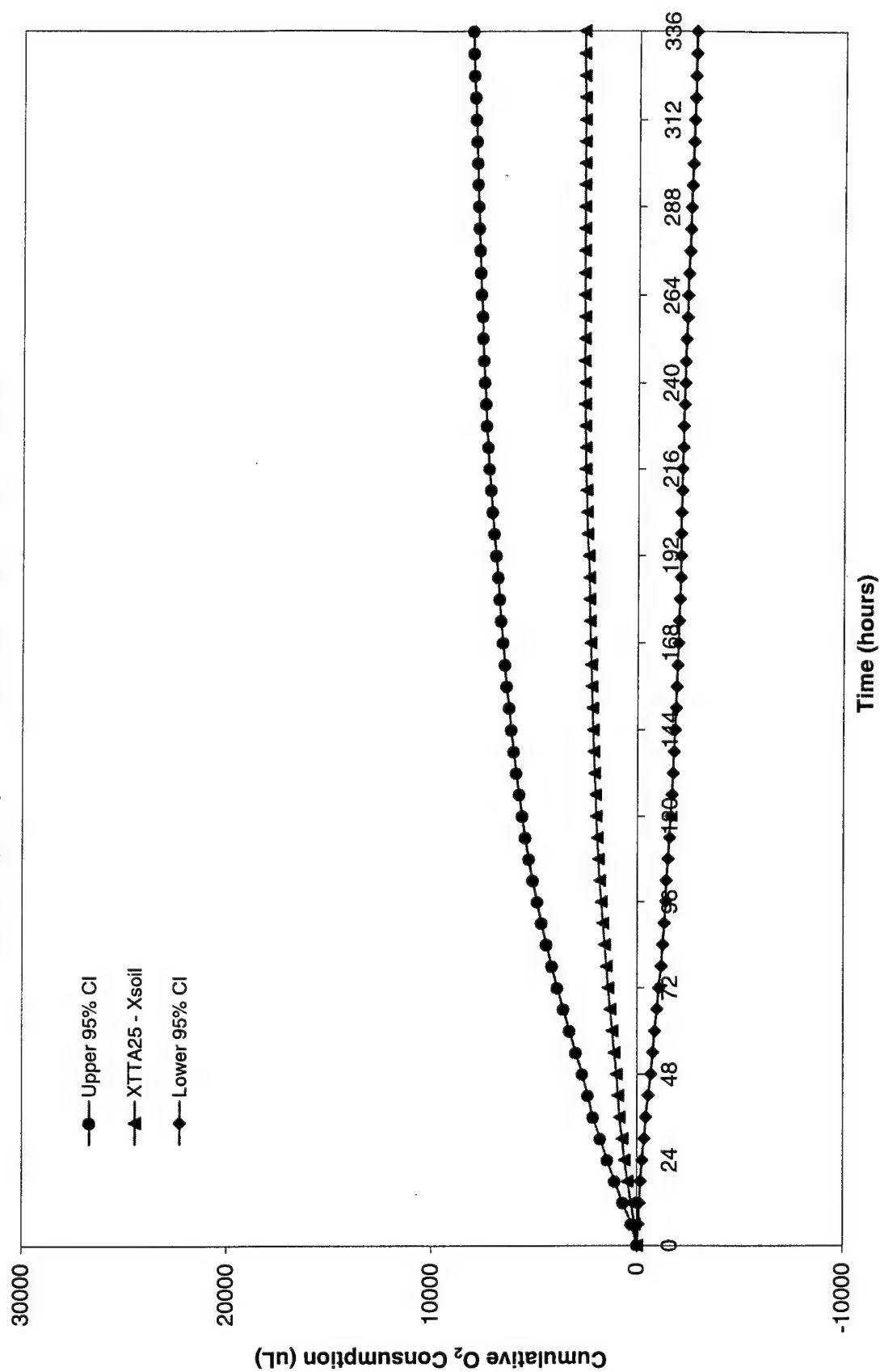


Table F-2 (Run-2) Data ( $O_2$ ) for Determining Biodegradation from the Individual Treatment of 250 mg/kg Tolytriazole on Uncontaminated Soil

| Time (hours) | Mean Soil (uL) | Std Dev Soil | Mean TTA250 in Soil (uL) | Std Dev TTA500 in Soil | Pooled Estimator $S_p^2$ | Standard Error | $X_{TTA250} - X_{Tol}$ | Calc T Value ( $T_{crit} = 2.447$ ) | Upper 95% CI | Lower 95% CI | Biodegradation /inhibition/ No effect |
|--------------|----------------|--------------|--------------------------|------------------------|--------------------------|----------------|------------------------|-------------------------------------|--------------|--------------|---------------------------------------|
| 0            | 0              | 0            | 0                        | 0                      | 0                        | 0              | 0                      | 0.000                               | 0            | 0            | NA                                    |
| 6            | 960            | 90           | 907                      | 80                     | 7002                     | 61             | -53                    | -0.865                              | 97           | -202         | no effect                             |
| 12           | 1502           | 146          | 1470                     | 135                    | 19217                    | 101            | -33                    | -0.323                              | 215          | -280         | no effect                             |
| 18           | 1894           | 201          | 1877                     | 185                    | 36265                    | 139            | -17                    | -0.123                              | 323          | -357         | no effect                             |
| 24           | 2180           | 243          | 2188                     | 225                    | 53433                    | 169            | 8                      | 0.048                               | 421          | -405         | no effect                             |
| 30           | 2414           | 268          | 2453                     | 258                    | 68171                    | 191            | 39                     | 0.204                               | 506          | -428         | no effect                             |
| 36           | 2649           | 285          | 2726                     | 291                    | 83561                    | 211            | 77                     | 0.366                               | 594          | -439         | no effect                             |
| 42           | 2857           | 292          | 2993                     | 322                    | 97718                    | 228            | 136                    | 0.595                               | 694          | -423         | no effect                             |
| 48           | 3038           | 296          | 3232                     | 352                    | 111489                   | 244            | 194                    | 0.795                               | 790          | -403         | no effect                             |
| 54           | 3196           | 296          | 3424                     | 377                    | 124088                   | 257            | 229                    | 0.889                               | 858          | -401         | no effect                             |
| 60           | 3347           | 297          | 3642                     | 403                    | 137446                   | 271            | 295                    | 1.089                               | 957          | -368         | no effect                             |
| 66           | 3483           | 300          | 3818                     | 425                    | 150249                   | 283            | 336                    | 1.185                               | 1028         | -357         | no effect                             |
| 72           | 3632           | 298          | 4002                     | 438                    | 157468                   | 290            | 371                    | 1.279                               | 1080         | -338         | no effect                             |
| 78           | 3817           | 294          | 4237                     | 461                    | 170386                   | 301            | 420                    | 1.393                               | 1157         | -318         | no effect                             |
| 84           | 4006           | 294          | 4468                     | 482                    | 183631                   | 313            | 482                    | 1.475                               | 1227         | -304         | no effect                             |
| 90           | 4198           | 291          | 4697                     | 492                    | 198830                   | 318            | 500                    | 1.571                               | 1278         | -279         | no effect                             |
| 96           | 4382           | 285          | 4923                     | 513                    | 202206                   | 328            | 541                    | 1.646                               | 1344         | -263         | no effect                             |
| 102          | 4557           | 279          | 5140                     | 529                    | 212849                   | 337            | 583                    | 1.730                               | 1407         | -242         | no effect                             |
| 108          | 4700           | 273          | 5321                     | 547                    | 224300                   | 346            | 621                    | 1.796                               | 1467         | -225         | no effect                             |
| 114          | 4843           | 269          | 5493                     | 563                    | 235025                   | 354            | 650                    | 1.836                               | 1516         | -216         | no effect                             |
| 120          | 4963           | 258          | 5625                     | 577                    | 244428                   | 361            | 682                    | 1.834                               | 1546         | -221         | no effect                             |
| 126          | 5090           | 250          | 5780                     | 589                    | 252258                   | 367            | 691                    | 1.883                               | 1588         | -207         | no effect                             |
| 132          | 5210           | 244          | 5922                     | 598                    | 258305                   | 371            | 712                    | 1.919                               | 1621         | -196         | no effect                             |
| 138          | 5334           | 236          | 6066                     | 610                    | 266503                   | 377            | 732                    | 1.941                               | 1654         | -191         | no effect                             |
| 144          | 5448           | 230          | 6200                     | 617                    | 271124                   | 380            | 752                    | 1.977                               | 1682         | -179         | no effect                             |
| 150          | 5548           | 220          | 6311                     | 630                    | 280341                   | 387            | 763                    | 1.974                               | 1710         | -183         | no effect                             |
| 156          | 5652           | 211          | 6439                     | 635                    | 283773                   | 389            | 787                    | 2.022                               | 1739         | -165         | no effect                             |
| 162          | 5762           | 202          | 6551                     | 642                    | 288370                   | 392            | 789                    | 2.012                               | 1749         | -171         | no effect                             |
| 168          | 5875           | 192          | 6682                     | 647                    | 291284                   | 394            | 808                    | 2.049                               | 1772         | -157         | no effect                             |
| 174          | 5960           | 185          | 6789                     | 655                    | 297631                   | 398            | 829                    | 2.079                               | 1803         | -146         | no effect                             |
| 180          | 6038           | 176          | 6882                     | 659                    | 299707                   | 400            | 844                    | 2.112                               | 1823         | -134         | no effect                             |
| 186          | 6117           | 163          | 6973                     | 665                    | 303815                   | 403            | 856                    | 2.126                               | 1841         | -129         | no effect                             |
| 192          | 6188           | 155          | 7055                     | 666                    | 303737                   | 402            | 867                    | 2.155                               | 1852         | -118         | no effect                             |
| 198          | 6251           | 148          | 7141                     | 675                    | 311043                   | 407            | 890                    | 2.185                               | 1886         | -107         | no effect                             |
| 204          | 6324           | 140          | 7221                     | 681                    | 316030                   | 411            | 897                    | 2.186                               | 1902         | -107         | no effect                             |
| 210          | 6389           | 131          | 7300                     | 691                    | 324156                   | 416            | 911                    | 2.190                               | 1928         | -107         | no effect                             |
| 216          | 6459           | 124          | 7370                     | 696                    | 328304                   | 418            | 911                    | 2.178                               | 1935         | -113         | no effect                             |
| 222          | 6532           | 116          | 7450                     | 703                    | 333838                   | 422            | 917                    | 2.174                               | 1950         | -115         | no effect                             |
| 228          | 6601           | 110          | 7531                     | 711                    | 340967                   | 426            | 931                    | 2.182                               | 1974         | -113         | no effect                             |
| 234          | 6669           | 105          | 7608                     | 719                    | 348592                   | 431            | 939                    | 2.177                               | 1994         | -117         | no effect                             |
| 240          | 6736           | 97           | 7688                     | 728                    | 356088                   | 436            | 952                    | 2.184                               | 2018         | -115         | no effect                             |
| 246          | 6800           | 90           | 7758                     | 735                    | 362956                   | 440            | 958                    | 2.177                               | 2035         | -119         | no effect                             |
| 252          | 6864           | 84           | 7832                     | 743                    | 370877                   | 445            | 968                    | 2.177                               | 2056         | -120         | no effect                             |
| 258          | 6921           | 81           | 7895                     | 751                    | 378442                   | 449            | 975                    | 2.170                               | 2074         | -125         | no effect                             |
| 264          | 6987           | 78           | 7964                     | 758                    | 385321                   | 453            | 977                    | 2.156                               | 2086         | -132         | no effect                             |

Table F-2 (Run-2) Data (O<sub>2</sub>) for Determining Biodegradation from the Individual Treatment of 250 mg/kg Tolytriazole on Uncontaminated Soil

| Time (hours) | Mean Soil (uL) | Std Dev Soil | Mean TTA250 in Soil (uL) | Std Dev TTA500 in Soil | Pooled Estimator S <sub>p</sub> <sup>2</sup> | Standard Error | X <sub>TTA500</sub> - X <sub>uol</sub> | Calc T Value (T <sub>crit</sub> = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /inhibition/ No effect |
|--------------|----------------|--------------|--------------------------|------------------------|--|----------------|--|--|--------------|--------------|---------------------------------------|
| 270          | 7053           | 74           | 8036                     | 766                    | 392818                                       | 458            | 983                                    | 2.147                                    | 2103         | -137         | no effect                             |
| 276          | 7114           | 73           | 8108                     | 770                    | 397438                                       | 460            | 994                                    | 2.160                                    | 2121         | -132         | no effect                             |
| 282          | 7176           | 65           | 8175                     | 777                    | 404158                                       | 464            | 999                                    | 2.152                                    | 2135         | -137         | no effect                             |
| 288          | 7232           | 61           | 8236                     | 786                    | 413350                                       | 470            | 1004                                   | 2.139                                    | 2153         | -145         | no effect                             |
| 294          | 7284           | 56           | 8296                     | 795                    | 422532                                       | 475            | 1012                                   | 2.132                                    | 2174         | -149         | no effect                             |
| 300          | 7336           | 53           | 8350                     | 802                    | 429812                                       | 479            | 1014                                   | 2.119                                    | 2186         | -157         | no effect                             |
| 306          | 7382           | 49           | 8398                     | 811                    | 439111                                       | 484            | 1016                                   | 2.099                                    | 2200         | -169         | no effect                             |
| 312          | 7439           | 44           | 8462                     | 817                    | 445275                                       | 487            | 1024                                   | 2.101                                    | 2216         | -169         | no effect                             |
| 318          | 7494           | 43           | 8525                     | 823                    | 451792                                       | 491            | 1031                                   | 2.099                                    | 2232         | -171         | no effect                             |
| 324          | 7549           | 47           | 8597                     | 829                    | 459385                                       | 495            | 1048                                   | 2.118                                    | 2260         | -163         | no effect                             |
| 330          | 7604           | 49           | 8658                     | 837                    | 467993                                       | 500            | 1054                                   | 2.109                                    | 2276         | -169         | no effect                             |
| 336          | 7654           | 53           | 8719                     | 843                    | 474905                                       | 503            | 1065                                   | 2.116                                    | 2296         | -167         | no effect                             |

**FIGURE F-2 Difference Between the Means ( $O_2$ ) and 95% CI for  
250 mg/kg Tolytriazole on Uncontaminated Soil**

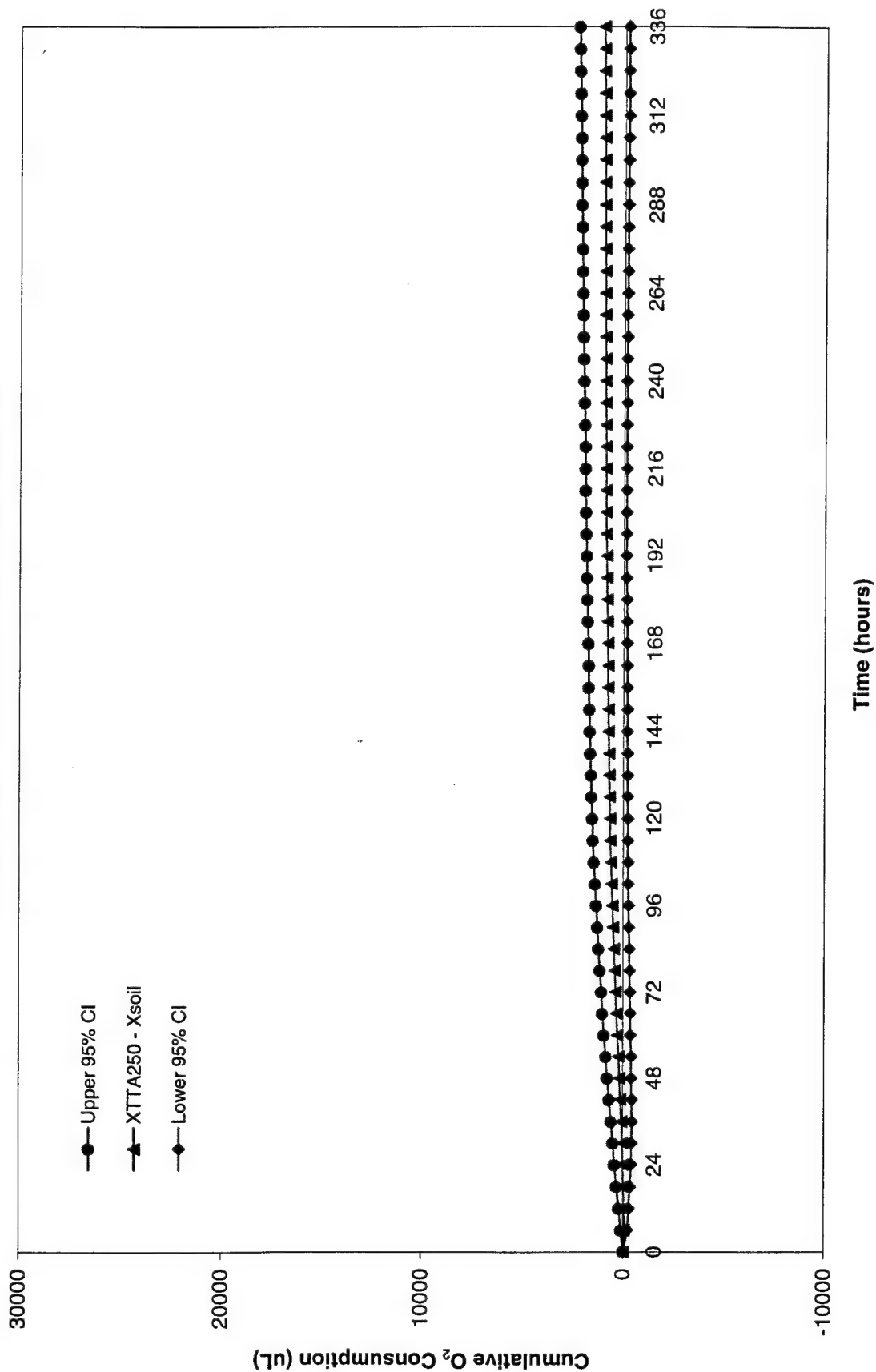


Table F-3 (Run-3) Data (O<sub>2</sub>) for Determining Biodegradation from the Individual Treatment of 500 mg/kg Tolytriazole on Uncontaminated Soil

| Time (hours) | Mean Soil (uL) | Std Dev Soil | Mean TTA500 In Soil (uL) | Std Dev TTA500 In Soil | Pooled Estimator S <sub>p</sub> <sup>2</sup> | Standard Error | X <sub>TTA500</sub> - X <sub>cal</sub> | Calc T Value (T <sub>crit</sub> = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /Inhibition/ No effect |
|--------------|----------------|--------------|--------------------------|------------------------|--|----------------|--|--|--------------|--------------|---------------------------------------|
| 0            | 0              | 0            | 0                        | 0                      | 0  | 0              | 0                                      | 0.000                                    | 0            | 0            | NA                                    |
| 6            | 610            | 25           | 683                      | 37                     | 1140   | 25             | 73                                     | 2.972                                    | 134          | 13           | Biodegradation                        |
| 12           | 981            | 29           | 1147                     | 84                     | 5027   | 52             | 166                                    | 3.198                                    | 292          | 39           | Biodegradation                        |
| 18           | 1258           | 34           | 1563                     | 123                    | 10398  | 74             | 305                                    | 4.096                                    | 487          | 123          | Biodegradation                        |
| 24           | 1493           | 36           | 1940                     | 158                    | 17075  | 95             | 447                                    | 4.684                                    | 680          | 213          | Biodegradation                        |
| 30           | 1725           | 43           | 2269                     | 189                    | 24411  | 114            | 544                                    | 4.768                                    | 823          | 265          | Biodegradation                        |
| 36           | 1949           | 48           | 2582                     | 220                    | 32978  | 133            | 634                                    | 4.777                                    | 958          | 309          | Biodegradation                        |
| 42           | 2169           | 35           | 2887                     | 247                    | 41113  | 148            | 718                                    | 4.849                                    | 1080         | 356          | Biodegradation                        |
| 48           | 2361           | 34           | 3161                     | 271                    | 49156  | 162            | 799                                    | 4.936                                    | 1195         | 403          | Biodegradation                        |
| 54           | 2544           | 40           | 3394                     | 295                    | 58353  | 176            | 850                                    | 4.817                                    | 1281         | 418          | Biodegradation                        |
| 60           | 2718           | 40           | 3631                     | 316                    | 67118  | 189            | 913                                    | 4.825                                    | 1376         | 450          | Biodegradation                        |
| 66           | 2878           | 48           | 3826                     | 337                    | 76580  | 202            | 948                                    | 4.689                                    | 1442         | 453          | Biodegradation                        |
| 72           | 3032           | 60           | 4053                     | 359                    | 86896  | 215            | 1020                                   | 4.740                                    | 1547         | 494          | Biodegradation                        |
| 78           | 3197           | 70           | 4232                     | 372                    | 93759  | 224            | 1035                                   | 4.629                                    | 1582         | 488          | Biodegradation                        |
| 84           | 3357           | 79           | 4417                     | 391                    | 103896                                       | 235            | 1080                                   | 4.504                                    | 1636         | 484          | Biodegradation                        |
| 90           | 3519           | 92           | 4590                     | 409                    | 114380                                       | 247            | 1071                                   | 4.334                                    | 1675         | 466          | Biodegradation                        |
| 96           | 3673           | 113          | 4779                     | 424                    | 124358                                       | 258            | 1106                                   | 4.295                                    | 1736         | 476          | Biodegradation                        |
| 102          | 3823           | 123          | 4957                     | 442                    | 135492                                       | 269            | 1134                                   | 4.219                                    | 1792         | 476          | Biodegradation                        |
| 108          | 3968           | 134          | 5117                     | 457                    | 145105                                       | 278            | 1149                                   | 4.129                                    | 1829         | 468          | Biodegradation                        |
| 114          | 4050           | 140          | 5210                     | 467                    | 151692                                       | 284            | 1150                                   | 4.044                                    | 1846         | 454          | Biodegradation                        |
| 120          | 4231           | 153          | 5428                     | 485                    | 164415                                       | 296            | 1197                                   | 4.042                                    | 1922         | 472          | Biodegradation                        |
| 126          | 4387           | 165          | 5630                     | 498                    | 174507                                       | 305            | 1242                                   | 4.073                                    | 1989         | 496          | Biodegradation                        |
| 132          | 4566           | 177          | 5850                     | 514                    | 186501                                       | 315            | 1284                                   | 4.072                                    | 2056         | 512          | Biodegradation                        |
| 138          | 4730           | 188          | 6053                     | 536                    | 203115                                       | 329            | 1323                                   | 4.021                                    | 2129         | 518          | Biodegradation                        |
| 144          | 4888           | 204          | 6256                     | 549                    | 214849                                       | 339            | 1368                                   | 4.041                                    | 2196         | 539          | Biodegradation                        |
| 150          | 5068           | 219          | 6468                     | 568                    | 230704                                       | 351            | 1400                                   | 3.991                                    | 2258         | 542          | Biodegradation                        |
| 156          | 5226           | 229          | 6641                     | 583                    | 243969                                       | 361            | 1415                                   | 3.922                                    | 2298         | 532          | Biodegradation                        |
| 162          | 5388           | 248          | 6822                     | 599                    | 260039                                       | 372            | 1433                                   | 3.849                                    | 2345         | 522          | Biodegradation                        |
| 168          | 5532           | 262          | 6996                     | 615                    | 274892                                       | 383            | 1464                                   | 3.822                                    | 2400         | 527          | Biodegradation                        |
| 174          | 5677           | 280          | 7150                     | 630                    | 290515                                       | 394            | 1473                                   | 3.743                                    | 2436         | 510          | Biodegradation                        |
| 180          | 5813           | 290          | 7290                     | 641                    | 301518                                       | 401            | 1477                                   | 3.684                                    | 2459         | 496          | Biodegradation                        |
| 186          | 5958           | 301          | 7448                     | 654                    | 315609                                       | 410            | 1490                                   | 3.631                                    | 2494         | 486          | Biodegradation                        |
| 192          | 6081           | 308          | 7572                     | 667                    | 327998                                       | 418            | 1491                                   | 3.565                                    | 2514         | 468          | Biodegradation                        |
| 198          | 6208           | 317          | 7704                     | 679                    | 341228                                       | 427            | 1496                                   | 3.507                                    | 2540         | 452          | Biodegradation                        |
| 204          | 6323           | 328          | 7817                     | 694                    | 356817                                       | 436            | 1494                                   | 3.425                                    | 2562         | 427          | Biodegradation                        |
| 210          | 6436           | 339          | 7929                     | 705                    | 369596                                       | 444            | 1493                                   | 3.362                                    | 2579         | 406          | Biodegradation                        |
| 216          | 6567           | 348          | 8081                     | 717                    | 383127                                       | 452            | 1515                                   | 3.351                                    | 2621         | 409          | Biodegradation                        |
| 222          | 6693           | 355          | 8213                     | 724                    | 390995                                       | 457            | 1520                                   | 3.328                                    | 2637         | 402          | Biodegradation                        |
| 228          | 6805           | 362          | 8329                     | 733                    | 401343                                       | 463            | 1525                                   | 3.295                                    | 2657         | 392          | Biodegradation                        |
| 234          | 6917           | 371          | 8438                     | 745                    | 416020                                       | 471            | 1520                                   | 3.228                                    | 2673         | 368          | Biodegradation                        |
| 240          | 7022           | 383          | 8570                     | 758                    | 432228                                       | 480            | 1548                                   | 3.223                                    | 2723         | 373          | Biodegradation                        |
| 246          | 7143           | 390          | 8679                     | 772                    | 447815                                       | 489            | 1536                                   | 3.143                                    | 2732         | 340          | Biodegradation                        |
| 252          | 7256           | 397          | 8808                     | 779                    | 457464                                       | 494            | 1550                                   | 3.137                                    | 2758         | 341          | Biodegradation                        |
| 258          | 7362           | 405          | 8923                     | 792                    | 472756                                       | 502            | 1560                                   | 3.107                                    | 2789         | 332          | Biodegradation                        |
| 264          | 7482           | 410          | 9065                     | 802                    | 484486                                       | 508            | 1582                                   | 3.113                                    | 2826         | 339          | Biodegradation                        |



Table F-3 (Run-3) Data (O<sub>2</sub>) for Determining Biodegradation from the Individual Treatment of 500 mg/kg Tolytriazole on Uncontaminated Soil

| Time (hours) | Mean Soil (uL) | Std Dev Soil | Mean TTA500 In Soil (uL) | Std Dev TTA500 In Soil | Pooled Estimator S <sub>p</sub> <sup>2</sup> | Standard Error | $X_{TTA500} - X_{NoEff}$ | Calc T Value (T <sub>crit</sub> = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /Inhibition/ No effect |
|--------------|----------------|--------------|--------------------------|------------------------|--|----------------|--------------------------|--|--------------|--------------|---------------------------------------|
| 270          | 7593           | 422          | 9189                     | 812                    | 498587                                       | 516            | 1596                     | 3.096                                    | 2858         | 335          | Biodegradation                        |
| 276          | 7688           | 432          | 9294                     | 823                    | 513552                                       | 523            | 1606                     | 3.068                                    | 2886         | 325          | Biodegradation                        |
| 282          | 7782           | 438          | 9379                     | 833                    | 526909                                       | 530            | 1597                     | 3.013                                    | 2894         | 300          | Biodegradation                        |
| 288          | 7881           | 448          | 9497                     | 845                    | 542917                                       | 538            | 1615                     | 3.002                                    | 2932         | 299          | Biodegradation                        |
| 294          | 7980           | 454          | 9612                     | 855                    | 556176                                       | 545            | 1632                     | 2.997                                    | 2965         | 299          | Biodegradation                        |
| 300          | 8081           | 462          | 9725                     | 862                    | 566064                                       | 549            | 1643                     | 2.991                                    | 2988         | 299          | Biodegradation                        |
| 306          | 8180           | 470          | 9837                     | 869                    | 577373                                       | 555            | 1657                     | 2.986                                    | 3015         | 299          | Biodegradation                        |
| 312          | 8277           | 482          | 9949                     | 882                    | 596556                                       | 564            | 1672                     | 2.964                                    | 3052         | 292          | Biodegradation                        |
| 318          | 8382           | 493          | 10065                    | 889                    | 608021                                       | 569            | 1683                     | 2.956                                    | 3077         | 290          | Biodegradation                        |
| 324          | 8476           | 500          | 10172                    | 899                    | 622033                                       | 576            | 1696                     | 2.944                                    | 3105         | 286          | Biodegradation                        |
| 330          | 8549           | 511          | 10279                    | 906                    | 633967                                       | 581            | 1730                     | 2.976                                    | 3153         | 308          | Biodegradation                        |
| 336          | 8624           | 514          | 10380                    | 912                    | 642748                                       | 585            | 1756                     | 2.999                                    | 3188         | 323          | Biodegradation                        |

**Figure F-3 Difference Between the Means ( $O_2$ ) and 95% CI for  
500 mg/kg Tolyltriazole on Uncontaminated Soil**

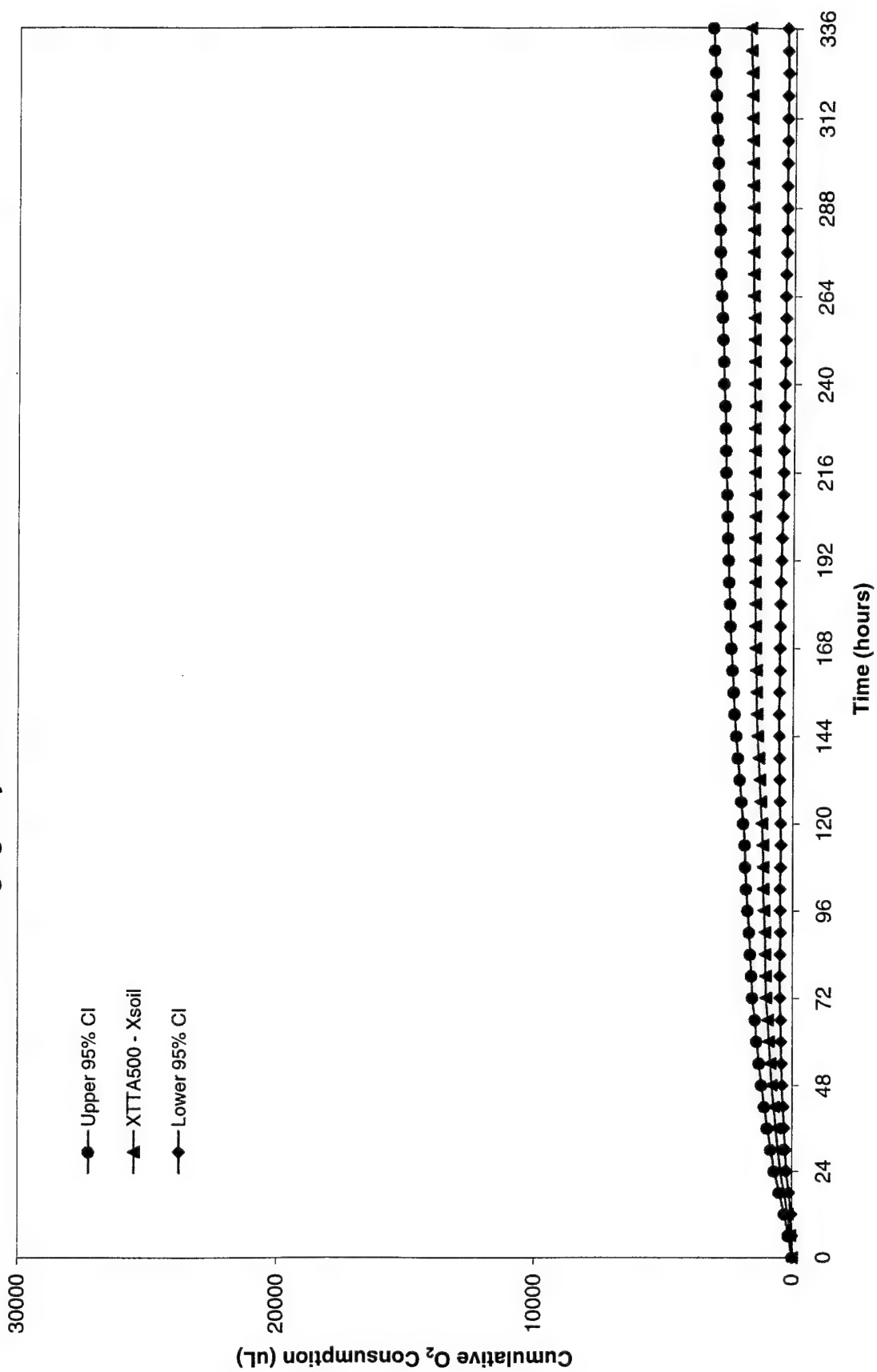


Table F-4 (Run-5) Data (O<sub>2</sub>) for Determining Biodegradation from the Individual Treatment of 750 mg/kg Tolytriazole on Uncontaminated Soil

| Time (hours) | Mean Soil (uL) | (RUN-3 Used) Std Dev Soil | Mean TTA750 in Soil (uL) | Std Dev TTA750 in Soil | Pooled Estimator S <sub>p</sub> <sup>2</sup> | Standard Error | X <sub>TTA750</sub> - X <sub>soil</sub> | Calc T Value (T <sub>crit</sub> = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /Inhibition/ No effect |
|--------------|----------------|---------------------------|--------------------------|------------------------|--|----------------|---|--|--------------|--------------|---------------------------------------|
| 0            | 0              | 0                         | 0                        | 0                      | 0  | 0              | 0                                       | 0.000                                    | 0            | 0            | NA                                    |
| 6            | 610            | 25                        | 553                      | 14                     | 342  | 14             | -56                                     | -4.174                                   | -23          | -89          | Biodegradation                        |
| 12           | 981            | 29                        | 950                      | 24                     | 657  | 19             | -31                                     | -1.640                                   | 15           | -76          | Biodegradation                        |
| 18           | 1258           | 34                        | 1306                     | 44                     | 1646   | 30             | 48                                      | 1.621                                    | 121          | -24          | Biodegradation                        |
| 24           | 1493           | 36                        | 1608                     | 60                     | 2844   | 39             | 115                                     | 2.956                                    | 210          | 20           | Biodegradation                        |
| 30           | 1725           | 43                        | 1913                     | 77                     | 4586   | 49             | 188                                     | 3.801                                    | 309          | 67           | Biodegradation                        |
| 36           | 1949           | 48                        | 2212                     | 93                     | 6547   | 59             | 263                                     | 4.450                                    | 408          | 118          | Biodegradation                        |
| 42           | 2169           | 35                        | 2505                     | 106                    | 7974   | 65             | 335                                     | 5.142                                    | 495          | 176          | Biodegradation                        |
| 48           | 2361           | 34                        | 2786                     | 110                    | 8470   | 67             | 434                                     | 6.464                                    | 599          | 270          | Biodegradation                        |
| 54           | 2544           | 40                        | 3075                     | 118                    | 9765   | 72             | 531                                     | 7.360                                    | 708          | 355          | Biodegradation                        |
| 60           | 2718           | 40                        | 3351                     | 124                    | 10750  | 76             | 633                                     | 8.354                                    | 818          | 447          | Biodegradation                        |
| 66           | 2878           | 48                        | 3613                     | 134                    | 12708  | 82             | 735                                     | 9.927                                    | 936          | 534          | Biodegradation                        |
| 72           | 3032           | 60                        | 3864                     | 140                    | 14216  | 87             | 831                                     | 10.529                                   | 1045         | 618          | Biodegradation                        |
| 78           | 3197           | 70                        | 4141                     | 142                    | 15089  | 90             | 944                                     | 10.890                                   | 1164         | 725          | Biodegradation                        |
| 84           | 3357           | 79                        | 4395                     | 150                    | 17038  | 95             | 1038                                    | 10.890                                   | 1271         | 805          | Biodegradation                        |
| 90           | 3519           | 92                        | 4658                     | 156                    | 19037  | 101            | 1139                                    | 11.304                                   | 1386         | 892          | Biodegradation                        |
| 96           | 3673           | 113                       | 4877                     | 160                    | 20837  | 105            | 1204                                    | 11.419                                   | 1462         | 946          | Biodegradation                        |
| 102          | 3823           | 123                       | 5098                     | 160                    | 22067  | 108            | 1276                                    | 11.759                                   | 1541         | 1010         | Biodegradation                        |
| 108          | 3968           | 134                       | 5329                     | 170                    | 25284  | 116            | 1361                                    | 11.724                                   | 1646         | 1077         | Biodegradation                        |
| 114          | 4060           | 140                       | 5531                     | 178                    | 27518  | 121            | 1471                                    | 12.144                                   | 1768         | 1175         | Biodegradation                        |
| 120          | 4231           | 153                       | 5739                     | 183                    | 30251  | 127            | 1508                                    | 11.872                                   | 1819         | 1197         | Biodegradation                        |
| 126          | 4387           | 165                       | 5910                     | 191                    | 33325  | 133            | 1523                                    | 11.422                                   | 1896         | 1201         | Biodegradation                        |
| 132          | 4566           | 177                       | 6115                     | 203                    | 37879  | 142            | 1549                                    | 10.895                                   | 1953         | 1210         | Biodegradation                        |
| 138          | 4730           | 188                       | 6311                     | 217                    | 43254  | 152            | 1581                                    | 10.412                                   | 1981         | 1193         | Biodegradation                        |
| 144          | 4888           | 204                       | 6475                     | 228                    | 48604  | 161            | 1587                                    | 9.858                                    | 1988         | 1152         | Biodegradation                        |
| 150          | 5068           | 219                       | 6639                     | 241                    | 54886  | 171            | 1570                                    | 9.193                                    | 2031         | 1149         | Biodegradation                        |
| 156          | 5226           | 229                       | 6816                     | 255                    | 60835  | 180            | 1590                                    | 8.829                                    | 2114         | 1170         | Biodegradation                        |
| 162          | 5388           | 248                       | 7030                     | 272                    | 69896  | 193            | 1642                                    | 8.504                                    | 2156         | 1178         | Biodegradation                        |
| 168          | 5532           | 262                       | 7195                     | 283                    | 76078  | 201            | 1666                                    | 8.255                                    | 2215         | 1180         | Biodegradation                        |
| 174          | 5677           | 280                       | 7373                     | 295                    | 84225  | 212            | 1696                                    | 8.003                                    | 2253         | 1159         | Biodegradation                        |
| 180          | 5813           | 290                       | 7530                     | 305                    | 90101  | 219            | 1717                                    | 7.831                                    | 2278         | 1147         | Biodegradation                        |
| 186          | 5958           | 301                       | 7668                     | 312                    | 95253  | 225            | 1710                                    | 7.587                                    | 2302         | 1144         | Biodegradation                        |
| 192          | 6081           | 308                       | 7793                     | 320                    | 100104                                       | 231            | 1721                                    | 7.411                                    | 2346         | 1157         | Biodegradation                        |
| 198          | 6208           | 317                       | 7929                     | 329                    | 105723                                       | 237            | 1721                                    | 7.247                                    | 2394         | 1148         | Biodegradation                        |
| 204          | 6323           | 328                       | 8068                     | 340                    | 113182                                       | 246            | 1745                                    | 7.103                                    | 2423         | 1164         | Biodegradation                        |
| 210          | 6436           | 339                       | 8212                     | 350                    | 119870                                       | 253            | 1776                                    | 7.022                                    | 2454         | 1154         | Biodegradation                        |
| 216          | 6567           | 348                       | 8352                     | 361                    | 127265                                       | 261            | 1836                                    | 6.854                                    | 2509         | 1164         | Biodegradation                        |
| 222          | 6693           | 355                       | 8493                     | 372                    | 134074                                       | 267            | 1844                                    | 6.681                                    | 2533         | 1154         | Biodegradation                        |
| 228          | 6805           | 362                       | 8641                     | 383                    | 141616                                       | 275            | 1844                                    | 6.539                                    | 2426         | 1033         | Biodegradation                        |
| 234          | 6917           | 371                       | 8761                     | 393                    | 149020                                       | 282            | 1706                                    | 6.397                                    | 2414         | 954          | Biodegradation                        |
| 240          | 7022           | 383                       | 8752                     | 393                    | 151867                                       | 289            | 1671                                    | 6.261                                    | 2388         | 926          | Biodegradation                        |
| 246          | 7143           | 390                       | 8849                     | 399                    | 156905                                       | 293            | 1667                                    | 6.105                                    | 2407         | 906          | Biodegradation                        |
| 252          | 7256           | 397                       | 8927                     | 403                    | 161105                                       | 303            | 1658                                    | 5.937                                    | 2410         |              | Biodegradation                        |
| 258          | 7362           | 405                       | 9029                     | 419                    | 171688                                       | 307            |   |  |              |              | Biodegradation                        |
| 264          | 7482           | 410                       | 9140                     | 426                    | 176969                                       | 307            |   |  |              |              | Biodegradation                        |

Table F-4 (Run-5) Data (O<sub>2</sub>) for Determining Biodegradation from the Individual Treatment of 750 mg/kg Tolytriazole on Uncontaminated Soil

| Time<br>(hours) | (RUN-3 Used)<br>Mean<br>Soil<br>(uL) | (RUN-3 Used)<br>Std Dev<br>Soil | Mean<br>TTA750 in Soil<br>(uL) | Std Dev<br>TTA750 in Soil | Pooled<br>Estimator<br>S <sub>p</sub> <sup>2</sup> | Standard<br>Error | X <sub>TTA750</sub> - X <sub>soil</sub> | Calc T Value<br>(T <sub>crit</sub> = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation<br>/inhibition/<br>No effect |
|-----------------|--------------------------------------|---------------------------------|--------------------------------|---------------------------|--|-------------------|---|---|--------------|--------------|---|
| 270             | 7593                                 | 422                             | 9257                           | 435                       | 185559   | 315               | 1664                                    | 5.289                                       | 2434         | 894          | Biodegradation                              |
| 276             | 7686                                 | 432                             | 9344                           | 442                       | 192173   | 320               | 1656                                    | 5.172                                       | 2439         | 873          | Biodegradation                              |
| 282             | 7682                                 | 438                             | 9452                           | 449                       | 198389   | 325               | 1670                                    | 5.133                                       | 2466         | 874          | Biodegradation                              |
| 288             | 7881                                 | 448                             | 9537                           | 455                       | 204497   | 330               | 1656                                    | 5.014                                       | 2464         | 848          | Biodegradation                              |
| 294             | 7980                                 | 454                             | 9635                           | 461                       | 210630   | 335               | 1655                                    | 4.938                                       | 2475         | 835          | Biodegradation                              |
| 300             | 8081                                 | 462                             | 9737                           | 470                       | 218816   | 342               | 1656                                    | 4.848                                       | 2492         | 820          | Biodegradation                              |
| 306             | 8180                                 | 470                             | 9835                           | 477                       | 225663   | 347               | 1655                                    | 4.770                                       | 2504         | 806          | Biodegradation                              |
| 312             | 8277                                 | 482                             | 9920                           | 484                       | 233501   | 353               | 1643                                    | 4.655                                       | 2506         | 779          | Biodegradation                              |
| 318             | 8382                                 | 493                             | 10032                          | 491                       | 241905   | 359               | 1651                                    | 4.596                                       | 2530         | 772          | Biodegradation                              |
| 324             | 8476                                 | 500                             | 10126                          | 498                       | 248862   | 364               | 1650                                    | 4.529                                       | 2542         | 759          | Biodegradation                              |
| 330             | 8549                                 | 511                             | 10215                          | 504                       | 256490   | 370               | 1666                                    | 4.504                                       | 2571         | 761          | Biodegradation                              |
| 336             | 8624                                 | 514                             | 10290                          | 512                       | 263235   | 375               | 1666                                    | 4.447                                       | 2583         | 749          | Biodegradation                              |

**Figure F-4 Difference Between the Means ( $O_2$ ) and 95% CI for  
750 mg/kg Tolyltriazole on Uncontaminated Soil**

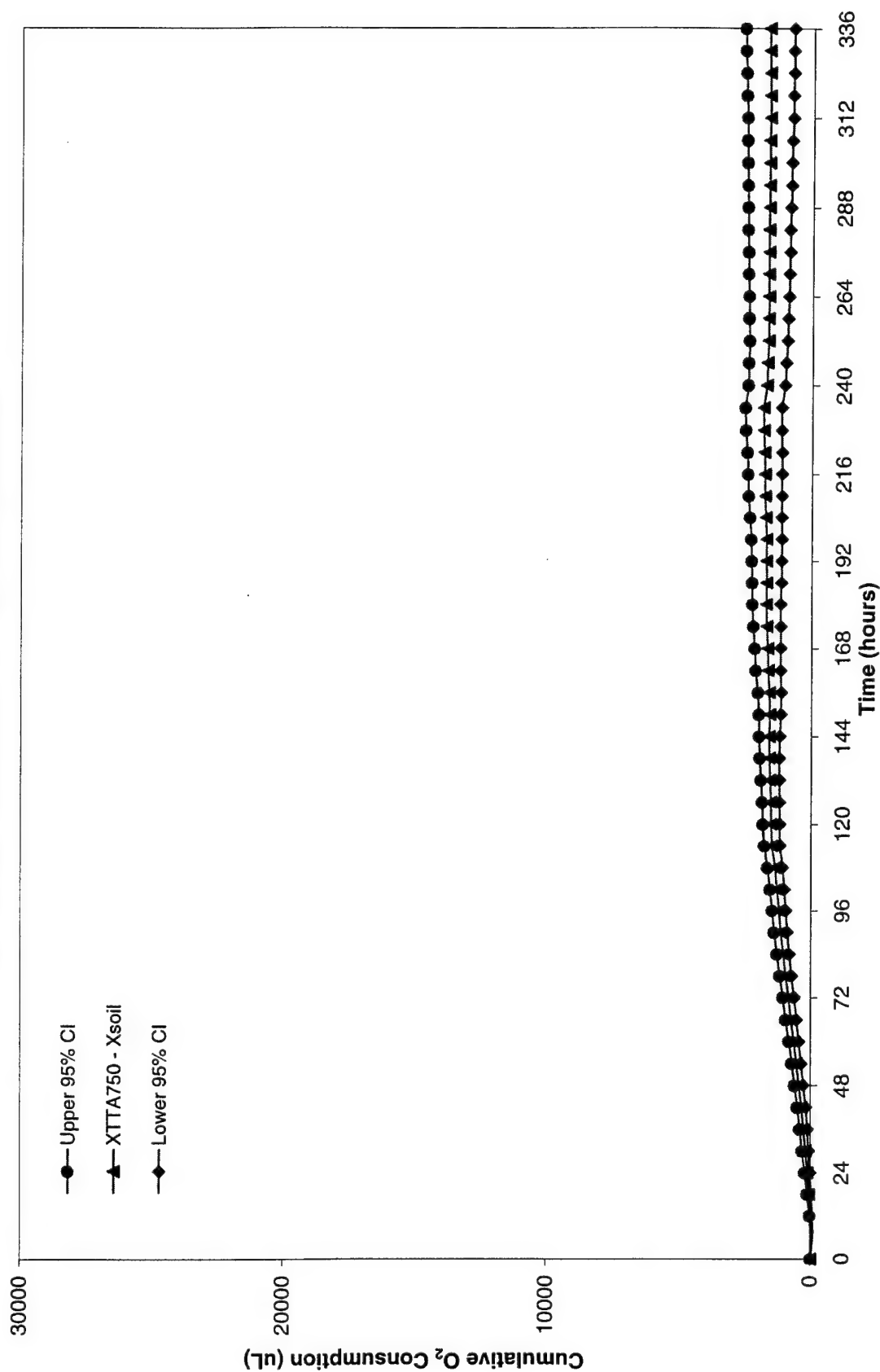


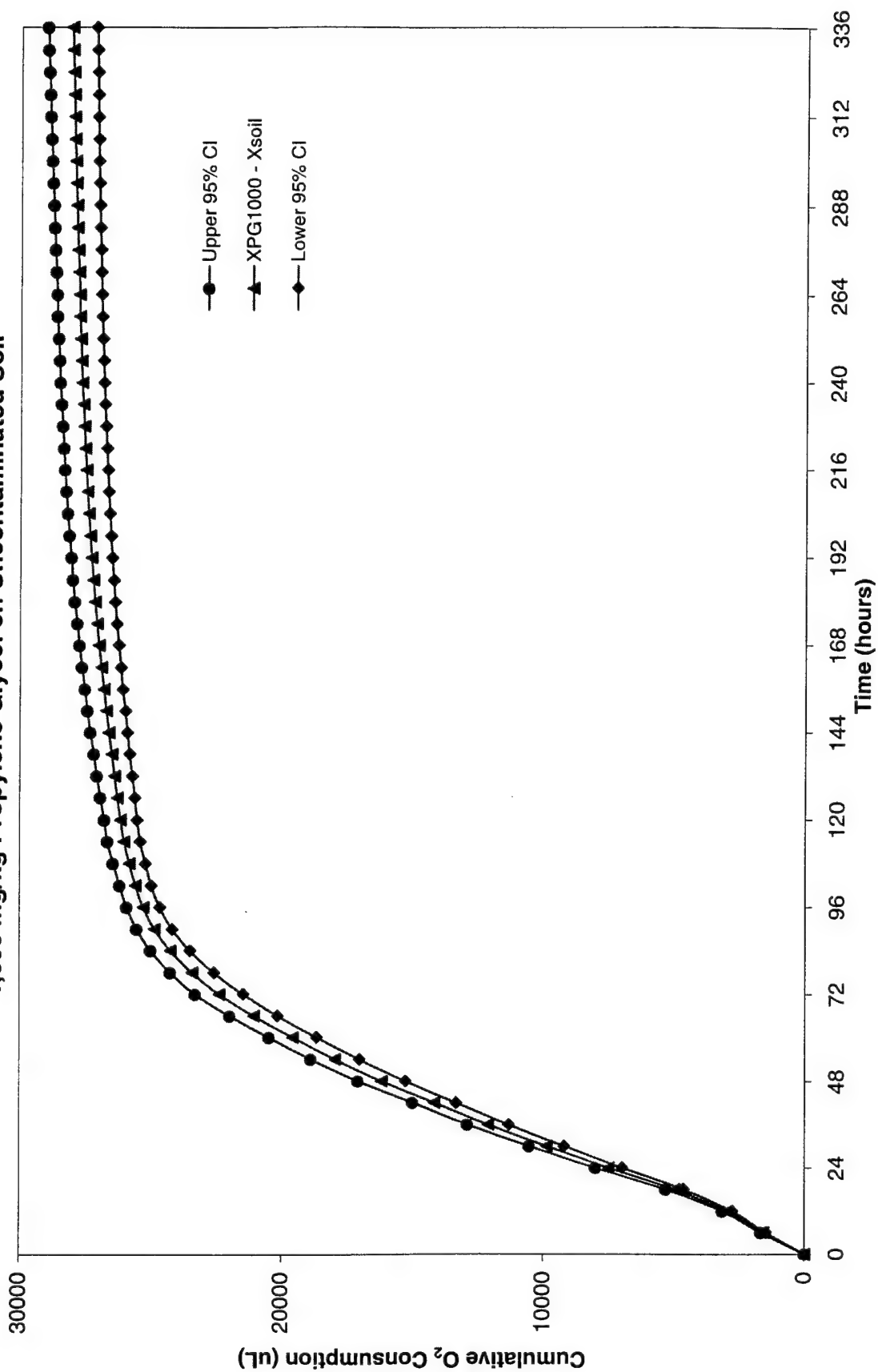
Table F-5 (Run-3) Data (O<sub>2</sub>) for Determining Biodegradation from Individual Treatment of 1,000 mg/kg Propylene Glycol on Uncontaminated Soil

| Time (hrs) | Mean Soil (uL) | Std Dev Soil | Mean PG1000 In Soil (uL) | Std Dev PG1000 In Soil | Pooled Estimator S <sub>p</sub> <sup>2</sup> | Standard Error | X <sub>Fat1000</sub> - X <sub>soil</sub> | Calc T Value (T <sub>crit</sub> = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation Inhibition/ No effect |
|------------|----------------|--------------|--------------------------|------------------------|--|----------------|--|--|--------------|--------------|--------------------------------------|
| 0          | 0              | 0            | 0                        | 0                      | 0  | 0              | 0  | 0.000                                    | 0            | 0            | N/A                                  |
| 6          | 610            | 25           | 2189                     | 61                     | 2715   | 38             | 1580                                     | 41.508                                   | 1673         | 1486         | Biodegradation                       |
| 12         | 981            | 29           | 3944                     | 124                    | 10591  | 75             | 2963                                     | 39.421                                   | 3147         | 2779         | Biodegradation                       |
| 18         | 1258           | 34           | 6240                     | 233                    | 36640  | 140            | 4982                                     | 35.641                                   | 5324         | 4640         | Biodegradation                       |
| 24         | 1493           | 36           | 8994                     | 354                    | 83807  | 211            | 7501                                     | 35.479                                   | 8018         | 6984         | Biodegradation                       |
| 30         | 1725           | 43           | 11593                    | 452                    | 136897                                       | 270            | 9668                                     | 36.520                                   | 10529        | 9207         | Biodegradation                       |
| 36         | 1949           | 48           | 14043                    | 539                    | 194723                                       | 322            | 12094                                    | 37.530                                   | 12883        | 11306        | Biodegradation                       |
| 42         | 2169           | 35           | 16312                    | 568                    | 215803                                       | 339            | 14142                                    | 41.686                                   | 14972        | 13312        | Biodegradation                       |
| 48         | 2361           | 34           | 18520                    | 622                    | 258077                                       | 371            | 16159                                    | 43.554                                   | 17066        | 15251        | Biodegradation                       |
| 54         | 2544           | 40           | 20489                    | 638                    | 271553                                       | 381            | 17945                                    | 47.154                                   | 18876        | 17014        | Biodegradation                       |
| 60         | 2718           | 40           | 22295                    | 624                    | 260307                                       | 373            | 19577                                    | 52.541                                   | 20489        | 18665        | Biodegradation                       |
| 66         | 2878           | 48           | 23960                    | 630                    | 265562                                       | 376            | 21082                                    | 56.017                                   | 22002        | 20161        | Biodegradation                       |
| 72         | 3032           | 60           | 25442                    | 630                    | 265505                                       | 376            | 22410                                    | 59.553                                   | 23331        | 21489        | Biodegradation                       |
| 78         | 3197           | 70           | 26650                    | 571                    | 219015                                       | 342            | 23453                                    | 68.622                                   | 24289        | 22617        | Biodegradation                       |
| 84         | 3357           | 79           | 27646                    | 513                    | 177705                                       | 308            | 24289                                    | 78.897                                   | 25042        | 23536        | Biodegradation                       |
| 90         | 3519           | 92           | 28411                    | 456                    | 141552                                       | 275            | 24892                                    | 90.595                                   | 25564        | 24220        | Biodegradation                       |
| 96         | 3673           | 113          | 28994                    | 421                    | 122396                                       | 255            | 25321                                    | 93.106                                   | 25946        | 24696        | Biodegradation                       |
| 102        | 3823           | 123          | 29441                    | 398                    | 110786                                       | 243            | 25618                                    | 105.392                                  | 26213        | 25023        | Biodegradation                       |
| 108        | 3998           | 134          | 29819                    | 407                    | 116417                                       | 249            | 25851                                    | 103.747                                  | 26461        | 25242        | Biodegradation                       |
| 114        | 4080           | 140          | 30113                    | 415                    | 121176                                       | 254            | 26053                                    | 102.482                                  | 26675        | 25431        | Biodegradation                       |
| 120        | 4231           | 153          | 30413                    | 414                    | 121862                                       | 255            | 26182                                    | 102.700                                  | 26806        | 25558        | Biodegradation                       |
| 126        | 4387           | 165          | 30698                    | 431                    | 132795                                       | 266            | 26309                                    | 98.858                                   | 26960        | 25658        | Biodegradation                       |
| 132        | 4566           | 177          | 30983                    | 444                    | 142109                                       | 275            | 26417                                    | 95.957                                   | 27091        | 25744        | Biodegradation                       |
| 138        | 4730           | 188          | 31253                    | 445                    | 144025                                       | 277            | 26523                                    | 95.699                                   | 27201        | 25845        | Biodegradation                       |
| 144        | 4888           | 204          | 31524                    | 460                    | 155137                                       | 288            | 26636                                    | 92.599                                   | 27340        | 25932        | Biodegradation                       |
| 150        | 5088           | 219          | 31806                    | 470                    | 162946                                       | 295            | 26738                                    | 90.699                                   | 27459        | 26016        | Biodegradation                       |
| 156        | 5226           | 229          | 32059                    | 466                    | 162143                                       | 284            | 26833                                    | 91.249                                   | 27553        | 26114        | Biodegradation                       |
| 162        | 5388           | 248          | 32318                    | 477                    | 172250                                       | 303            | 26930                                    | 88.849                                   | 27671        | 26188        | Biodegradation                       |
| 168        | 5532           | 262          | 32553                    | 478                    | 175225                                       | 306            | 27021                                    | 88.389                                   | 27769        | 26273        | Biodegradation                       |
| 174        | 5677           | 280          | 32777                    | 475                    | 176759                                       | 307            | 27100                                    | 88.265                                   | 27852        | 26349        | Biodegradation                       |
| 180        | 5813           | 290          | 32992                    | 482                    | 182957                                       | 312            | 27179                                    | 87.008                                   | 27943        | 26415        | Biodegradation                       |
| 186        | 5958           | 301          | 33204                    | 489                    | 189888                                       | 318            | 27245                                    | 85.614                                   | 28024        | 26467        | Biodegradation                       |
| 192        | 6081           | 308          | 33369                    | 487                    | 189993                                       | 318            | 27308                                    | 85.788                                   | 28087        | 26529        | Biodegradation                       |
| 198        | 6208           | 317          | 33576                    | 493                    | 195714                                       | 323            | 27368                                    | 84.710                                   | 28159        | 26578        | Biodegradation                       |
| 204        | 6323           | 328          | 33754                    | 497                    | 200726                                       | 327            | 27431                                    | 83.838                                   | 28232        | 26631        | Biodegradation                       |
| 210        | 6436           | 339          | 33919                    | 495                    | 201449                                       | 328            | 27482                                    | 83.844                                   | 28285        | 26680        | Biodegradation                       |
| 216        | 6567           | 348          | 34093                    | 501                    | 207508                                       | 333            | 27526                                    | 82.724                                   | 28340        | 26712        | Biodegradation                       |
| 222        | 6693           | 355          | 34261                    | 501                    | 209329                                       | 334            | 27568                                    | 82.507                                   | 28386        | 26750        | Biodegradation                       |
| 228        | 6805           | 362          | 34414                    | 498                    | 208857                                       | 334            | 27609                                    | 82.724                                   | 28426        | 26793        | Biodegradation                       |
| 234        | 6917           | 371          | 34570                    | 500                    | 212816                                       | 337            | 27653                                    | 82.081                                   | 28478        | 26829        | Biodegradation                       |
| 240        | 7022           | 383          | 34717                    | 502                    | 218076                                       | 341            | 27723                                    | 81.291                                   | 28558        | 26889        | Biodegradation                       |
| 246        | 7143           | 390          | 34867                    | 501                    | 217086                                       | 340            | 27695                                    | 81.392                                   | 28527        | 26862        | Biodegradation                       |
| 252        | 7256           | 397          | 35012                    | 502                    | 220873                                       | 343            | 27756                                    | 80.870                                   | 28596        | 26918        | Biodegradation                       |
| 258        | 7362           | 405          | 35154                    | 505                    | 224457                                       | 346            | 27791                                    | 80.323                                   | 28638        | 26945        | Biodegradation                       |
| 264        | 7482           | 410          | 35290                    | 501                    | 223379                                       | 345            | 27808                                    | 80.565                                   | 28652        | 26963        | Biodegradation                       |

Table F-5 (Run-3) Data (O<sub>2</sub>) for Determining Biodegradation from Individual Treatment of 1,000 mg/kg Propylene Glycol on Uncontaminated Soil

| Time (hrs) | Mean Soil (uL) | Std Dev Soil | Mean PG1000 In Soil (uL) | Std Dev PG1000 In Soil | Pooled Estimator S <sub>p</sub> <sup>2</sup> | Standard Error | X <sub>PG1000</sub> - X <sub>uL</sub> | Calc T Value (T <sub>crit</sub> = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /inhibition/ No effect |
|------------|----------------|--------------|--------------------------|------------------------|--|----------------|---------------------------------------|--|--------------|--------------|---------------------------------------|
| 270        | 7593           | 422          | 35428                    | 504                    | 228524                                       | 349            | 27835                                 | 79.731                                   | 28689        | 26981        | Biodegradation                        |
| 276        | 7688           | 432          | 35554                    | 505                    | 231944                                       | 352            | 27866                                 | 79.229                                   | 28727        | 27005        | Biodegradation                        |
| 282        | 7782           | 438          | 35678                    | 504                    | 233487                                       | 353            | 27895                                 | 79.050                                   | 28759        | 27032        | Biodegradation                        |
| 288        | 7881           | 448          | 35803                    | 505                    | 236631                                       | 355            | 27921                                 | 78.596                                   | 28791        | 27052        | Biodegradation                        |
| 294        | 7980           | 454          | 35926                    | 507                    | 239914                                       | 358            | 27946                                 | 78.126                                   | 28822        | 27071        | Biodegradation                        |
| 300        | 8081           | 462          | 36050                    | 507                    | 242584                                       | 360            | 27969                                 | 77.758                                   | 28849        | 27089        | Biodegradation                        |
| 306        | 8180           | 470          | 36171                    | 511                    | 247907                                       | 364            | 27992                                 | 76.981                                   | 28881        | 27102        | Biodegradation                        |
| 312        | 8277           | 482          | 36296                    | 514                    | 253534                                       | 368            | 28019                                 | 76.197                                   | 28919        | 27119        | Biodegradation                        |
| 318        | 8382           | 493          | 36419                    | 513                    | 256361                                       | 370            | 28038                                 | 75.826                                   | 28943        | 27133        | Biodegradation                        |
| 324        | 8476           | 500          | 36530                    | 518                    | 262094                                       | 374            | 28055                                 | 75.037                                   | 28969        | 27140        | Biodegradation                        |
| 330        | 8549           | 511          | 36630                    | 522                    | 268881                                       | 379            | 28081                                 | 74.155                                   | 29008        | 27155        | Biodegradation                        |
| 336        | 8624           | 514          | 36729                    | 520                    | 268144                                       | 378            | 28105                                 | 74.319                                   | 29030        | 27180        | Biodegradation                        |

**Figure F-5 Difference Between the Means ( $O_2$ ) and 95% CI for  
1,000 mg/kg Propylene Glycol on Uncontaminated Soil**





## Appendix G: Statistical Procedures for Determining whether or not Measurable Biodegradation Occurred from the Combined ADF Chemicals (Propylene Glycol with Tolyltriazole) on Uncontaminated Soil

The data listed in the following five tables and figures explains the possible types of (decreased/no influence/increased) on biodegradation from a combination of chemical components (PG with TTA) on uncontaminated soil. This determination was made by comparing the O<sub>2</sub> consumption of the soil contaminated with both PG and TTA against the soil contaminated with PG only and TTA only. A two-sample t-test was performed using a significance level of  $\alpha = 0.05$ . A 95% CI was developed from the t-test results to depict the O<sub>2</sub> consumption effects. Both populations were assumed to be normal and the two population variances were assumed to be equal.

H<sub>0</sub>: There was no effect on the O<sub>2</sub> consumption due to combining the two contaminants

H<sub>a</sub>: There was an effect (decreased or increased) on the O<sub>2</sub> consumption due to the two contaminants

The pooled estimator, which was an estimate of the common population variance was determined by using the following equation (Devore, 358):

$$S_p^2 = \frac{(n_1-1)*S_1^2 + (n_2-1)*S_2^2 + (n_3-1)*S_3^2 + (n_4-1)*S_4^2}{(n_1 + n_2 + n_3 + n_4) - 2}$$

Where n<sub>1</sub> through n<sub>n</sub> are the sample sizes of the respective treatments, and S<sub>1</sub> through S<sub>n</sub> are the standard deviations of the respective treatments.

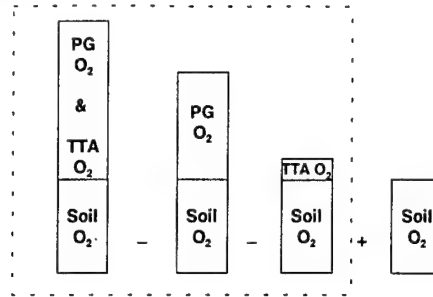
The standard error was determined by the following equations (Devore, 358):

$$\text{Std-Error} = S_p (1/n_1 + 1/n_2 + 1/n_3 + 1/n_4)^{1/2}$$

The calculated t-statistic (t) was then determined by dividing the difference of the means by the standard error.

$$t = \frac{(X_{\text{PG\&TTA}} - X_{\text{TTA}} - X_{\text{PG}}) + X_{\text{soil}}}{(\text{Std-Error})}$$

Shown below, is a visual depiction of the t-test and CI set-up with the O<sub>2</sub> mean totals.



This set-up provides a comparison of just the combined affects to be compared to the individual affects of ADF componts on soil.

The t-critical ( $t_{crit}$ ) was determined for a two-tailed test since the effects on biodegradation may be enhanced or inhibited as the alternate hypothesis, thus  $\alpha/2$  was used.

$$t_{crit} = t_{\alpha/2, (n_1+n_2+n_3+n_4) - 2} = 2.201 \text{ (Devore, 707)}$$

Given:  $\alpha = 0.05$

$n_1 = 3$  (number blank microcosms)

$n_2 = 5$  (number PG only microcosms)

$n_3 = 5$  (number TTA only microcosms)

$n_4 = 5$  (number PG & TTA microcosms)

The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical. An example of the test statistic is shown below:

|                    |                 |                |
|--------------------|-----------------|----------------|
| $t \leq -t_{crit}$ | $t \leq -2.201$ | Inhibition     |
| $t \geq t_{crit}$  | $t \geq 2.201$  | Biodegradation |

The t-critical ( $t_{crit}$ ) was determined for a two-tailed test since the effects on biodegradation may be enhanced or inhibited as the alternate hypothesis. The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical.

The upper and lower 95% confidence intervals were determined by using the following equation [Devore, 361]. This data was shown with the difference of the means (for the sample at its particular position on the time line) in Figures F-1 through F-4.

$$(X_{PG\&TTA} - X_{TTA} - X_{PG}) + X_{Soil} \pm (t_{\alpha/2, (n_1+n_2+n_3+n_4) - 2}) * (S_p) * (1/n_1 + 1/n_2 + 1/n_3 + 1/n_4)^{1/2}$$

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Table G-1 (Run-1) Data (O<sub>2</sub>) for Determining Biodegradation of 25 mg/kg Tolytriazole and 1,000 mg/kg Propylene Glycol

| Time (hours) | Mean Blank Soil | Std Dev Blank Soil | Mean TTA25 | Std Dev TTA25 | Mean PG1000 | Std Dev PG1000 | Mean TTA25 | Std Dev PG1000 | Pooled Estimator | Std Error | $\lambda_{TASPG1000} - \lambda_{TASPG1000} + X_{PG1000} - X_{cell}$ | Calc T Value (Tcrit = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /Inhibition/ No effect |
|--------------|-----------------|--------------------|------------|---------------|-------------|----------------|------------|----------------|------------------|-----------|---|------------------------------|--------------|--------------|---------------------------------------|
| 0            | 0               | 0                  | 0          | 0             | 0           | 0              | 0          | 0              | 0                | 0         | 0   | 0.000                        | 0            | 0            | N/A                                   |
| 6            | 495             | 56                 | 603        | 104           | 1993        | 83             | 1729       | 59             | 5687             | 73        | -372  | -5.103                       | -194         | -550         | Inhibition                            |
| 12           | 818             | 72                 | 1100       | 270           | 3716        | 160            | 3266       | 119            | 28867            | 164       | -732  | -4.458                       | -330         | -1133        | Inhibition                            |
| 18           | 1075            | 79                 | 1529       | 422           | 5719        | 269            | 5176       | 201            | 73538            | 262       | -997  | -3.804                       | -356         | -1638        | Inhibition                            |
| 24           | 1309            | 82                 | 1908       | 569           | 7695        | 436            | 7162       | 287            | 150087           | 374       | -1132   | -3.025                       | -216         | -2048        | Inhibition                            |
| 30           | 1548            | 90                 | 2263       | 730           | 9681        | 604            | 9141       | 374            | 260268           | 493       | -1256   | -2.547                       | -50          | -2462        | Inhibition                            |
| 36           | 1769            | 101                | 2620       | 876           | 11320       | 765            | 10818      | 434            | 385542           | 601       | -1353   | -2.253                       | 117          | -2823        | No effect                             |
| 42           | 1926            | 112                | 2847       | 1002          | 12693       | 985            | 12194      | 486            | 554551           | 719       | -1421   | -1.976                       | 339          | -3182        | No effect                             |
| 48           | 2049            | 113                | 3047       | 1138          | 14181       | 1261           | 13658      | 545            | 797170           | 863       | -1522   | -1.764                       | 589          | -3632        | No effect                             |
| 54           | 2240            | 114                | 3349       | 1281          | 15874       | 1525           | 15343      | 633            | 1093298          | 1010      | -1639   | -1.623                       | 832          | -4111        | No effect                             |
| 60           | 2431            | 112                | 3645       | 1417          | 17539       | 1785           | 16975      | 716            | 1428456          | 1155      | -1778   | -1.540                       | 1047         | -4604        | No effect                             |
| 66           | 2619            | 113                | 3929       | 1556          | 19109       | 2066           | 18536      | 799            | 1834090          | 1308      | -1884   | -1.440                       | 1318         | -5085        | No effect                             |
| 72           | 2797            | 119                | 4212       | 1690          | 20651       | 2292           | 20037      | 865            | 2216207          | 1438      | -2029   | -1.411                       | 1491         | -5548        | No effect                             |
| 78           | 2986            | 126                | 4477       | 1819          | 22161       | 2439           | 21513      | 910            | 2522896          | 1534      | -2140   | -1.394                       | 1615         | -5894        | No effect                             |
| 84           | 3186            | 132                | 4774       | 1934          | 23588       | 2576           | 22972      | 941            | 2818298          | 1622      | -2204   | -1.359                       | 1765         | -6172        | No effect                             |
| 90           | 3362            | 138                | 5033       | 2040          | 24962       | 2679           | 24350      | 976            | 3074783          | 1694      | -2283   | -1.347                       | 1863         | -6428        | No effect                             |
| 96           | 3518            | 143                | 5259       | 2136          | 26249       | 2747           | 25696      | 1003           | 3281336          | 1750      | -2294   | -1.311                       | 1988         | -6576        | No effect                             |
| 102          | 3709            | 147                | 5547       | 2226          | 27464       | 2792           | 27021      | 1026           | 3453061          | 1795      | -2280   | -1.270                       | 2113         | -6673        | No effect                             |
| 108          | 3880            | 151                | 5779       | 2313          | 28636       | 2783           | 28271      | 1044           | 3548439          | 1820      | -2264   | -1.244                       | 2189         | -6717        | No effect                             |
| 114          | 4058            | 157                | 6015       | 2396          | 29718       | 2736           | 29424      | 1071           | 3597281          | 1832      | -2250   | -1.228                       | 2234         | -6734        | No effect                             |
| 120          | 4238            | 162                | 6239       | 2476          | 30656       | 2683           | 30504      | 1083           | 3618018          | 1838      | -2154   | -1.172                       | 2342         | -6651        | No effect                             |
| 126          | 4428            | 168                | 6477       | 2545          | 31507       | 2609           | 31458      | 1084           | 3618007          | 1839      | -2098   | -1.142                       | 2399         | -6595        | No effect                             |
| 132          | 4622            | 172                | 6719       | 2611          | 32242       | 2538           | 32297      | 1105           | 3623286          | 1839      | -2042   | -1.110                       | 2458         | -6542        | No effect                             |
| 138          | 4808            | 176                | 6940       | 2673          | 32840       | 2519           | 32996      | 1116           | 3688036          | 1855      | -1976   | -1.065                       | 2564         | -6516        | No effect                             |
| 144          | 4996            | 182                | 7165       | 2730          | 33356       | 2525           | 33581      | 1112           | 3770835          | 1876      | -1945   | -1.037                       | 2645         | -6536        | No effect                             |
| 150          | 5181            | 183                | 7389       | 2783          | 33812       | 2551           | 34056      | 1118           | 3879615          | 1903      | -1964   | -1.032                       | 2692         | -6621        | No effect                             |
| 156          | 5366            | 188                | 7612       | 2837          | 34202       | 2612           | 34483      | 1112           | 4031786          | 1940      | -1965   | -1.013                       | 2782         | -6712        | No effect                             |
| 162          | 5534            | 189                | 7808       | 2882          | 34546       | 2695           | 34859      | 1103           | 4201236          | 1980      | -1962   | -0.991                       | 2884         | -6807        | No effect                             |
| 168          | 5705            | 196                | 8013       | 2925          | 34868       | 2779           | 35215      | 1101           | 4378675          | 2022      | -1961   | -0.970                       | 2986         | -6908        | No effect                             |
| 174          | 5862            | 203                | 8204       | 2966          | 35169       | 2867           | 35541      | 1108           | 4567168          | 2065      | -1970   | -0.954                       | 3082         | -7023        | No effect                             |
| 180          | 6015            | 206                | 8379       | 3009          | 35458       | 2950           | 35850      | 1108           | 4751375          | 2106      | -1972   | -0.937                       | 3181         | -7125        | No effect                             |
| 186          | 6151            | 215                | 8531       | 3048          | 35734       | 3031           | 36140      | 1108           | 4931992          | 2146      | -1975   | -0.920                       | 3275         | -7225        | No effect                             |
| 192          | 6277            | 219                | 8700       | 3083          | 35979       | 3110           | 36412      | 1115           | 5111524          | 2184      | -1990   | -0.911                       | 3355         | -7335        | No effect                             |
| 198          | 6452            | 233                | 8928       | 3119          | 36271       | 3203           | 36742      | 1132           | 5324101          | 2229      | -2005   | -0.900                       | 3449         | -7460        | No effect                             |
| 204          | 6595            | 237                | 9103       | 3153          | 36541       | 3288           | 37023      | 1144           | 5523111          | 2270      | -2025   | -0.892                       | 3531         | -7581        | No effect                             |
| 210          | 6743            | 247                | 9271       | 3189          | 36806       | 3372           | 37310      | 1152           | 5725192          | 2312      | -2024   | -0.876                       | 3632         | -7681        | No effect                             |
| 216          | 6888            | 259                | 9461       | 3222          | 37120       | 3573           | 37600      | 1166           | 6135462          | 2393      | -2093   | -0.875                       | 3763         | -7949        | No effect                             |
| 222          | 7016            | 264                | 9595       | 3256          | 37374       | 3651           | 37845      | 1174           | 6335600          | 2432      | -2108   | -0.867                       | 3842         | -8058        | No effect                             |
| 228          | 7149            | 267                | 9755       | 3288          | 37613       | 3733           | 38092      | 1189           | 6548287          | 2472      | -2127   | -0.860                       | 3923         | -8176        | No effect                             |
| 234          | 7278            | 268                | 9891       | 3314          | 37841       | 3807           | 38327      | 1201           | 6737777          | 2508      | -2127   | -0.848                       | 4009         | -8264        | No effect                             |

Table G-1 (Run-1) Data (O<sub>2</sub>) for Determining Biodegradation of 25 mg/kg Tolytriazole and 1,000 mg/kg Propylene Glycol

| Time (hours) | Mean Blank Soil | Std Dev Blank Soil | Mean TTA25 | Std Dev TTA25 | Mean PG1000 | Std Dev PG1000 | Mean  | Std Dev | Pooled Estimator | Std Error | $X_{TTA25PG1000} - X_{PG1000} + X_{soil}$ | Calc T Value (Tcrit = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /Inhibition/ No effect |
|--------------|-----------------|--------------------|------------|---------------|-------------|----------------|-------|---------|------------------|-----------|---|------------------------------|--------------|--------------|---------------------------------------|
| 240          | 7404            | 272                | 10034      | 3341          | 38057       | 3874           | 38557 | 1203    | 6914965          | 2540      | -2129                                     | -0.838                       | 4087         | -8346        | No effect                             |
| 246          | 7517            | 276                | 10157      | 3369          | 38267       | 3941           | 38776 | 1208    | 7096610          | 2574      | -2131                                     | -0.828                       | 4167         | -8428        | No effect                             |
| 252          | 7627            | 282                | 10271      | 3398          | 38469       | 4006           | 38981 | 1215    | 7276666          | 2606      | -2132                                     | -0.818                       | 4245         | -8510        | No effect                             |
| 258          | 7739            | 286                | 10374      | 3424          | 38684       | 4067           | 39175 | 1223    | 7450559          | 2637      | -2123                                     | -0.805                       | 4329         | -8576        | No effect                             |
| 264          | 7849            | 288                | 10508      | 3447          | 38850       | 4130           | 39382 | 1228    | 7622439          | 2667      | -2127                                     | -0.797                       | 4400         | -8654        | No effect                             |
| 270          | 7962            | 297                | 10619      | 3475          | 39038       | 4184           | 39583 | 1238    | 7786689          | 2696      | -2112                                     | -0.783                       | 4485         | -8710        | No effect                             |
| 276          | 8066            | 308                | 10721      | 3500          | 39221       | 4236           | 39773 | 1244    | 7947120          | 2723      | -2103                                     | -0.772                       | 4561         | -8768        | No effect                             |
| 282          | 8166            | 314                | 10819      | 3527          | 39388       | 4281           | 39946 | 1257    | 8097538          | 2749      | -2094                                     | -0.762                       | 4633         | -8822        | No effect                             |
| 288          | 8264            | 320                | 10922      | 3551          | 39551       | 4325           | 40125 | 1261    | 8238222          | 2773      | -2084                                     | -0.751                       | 4702         | -8869        | No effect                             |
| 294          | 8369            | 331                | 11016      | 3575          | 39705       | 4352           | 40290 | 1271    | 8346589          | 2791      | -2072                                     | -0.742                       | 4758         | -8902        | No effect                             |
| 300          | 8462            | 337                | 11117      | 3598          | 39855       | 4372           | 40466 | 1281    | 8440227          | 2807      | -2044                                     | -0.728                       | 4824         | -8912        | No effect                             |
| 306          | 8555            | 343                | 11207      | 3622          | 39992       | 4386           | 40622 | 1291    | 8521104          | 2820      | -2023                                     | -0.717                       | 4877         | -8924        | No effect                             |
| 312          | 8643            | 355                | 11302      | 3645          | 40125       | 4399           | 40782 | 1295    | 8594021          | 2832      | -2002                                     | -0.707                       | 4929         | -8932        | No effect                             |
| 318          | 8727            | 360                | 11386      | 3669          | 40252       | 4412           | 40925 | 1306    | 8672948          | 2845      | -1986                                     | -0.698                       | 4976         | -8948        | No effect                             |
| 324          | 8820            | 370                | 11501      | 3691          | 40387       | 4424           | 41092 | 1318    | 8749728          | 2858      | -1977                                     | -0.692                       | 5016         | -8969        | No effect                             |
| 330          | 8910            | 373                | 11598      | 3712          | 40512       | 4435           | 41249 | 1321    | 8814134          | 2868      | -1952                                     | -0.680                       | 5067         | -8970        | No effect                             |
| 336          | 8992            | 373                | 11684      | 3732          | 40628       | 4447           | 41397 | 1326    | 8882142          | 2879      | -1923                                     | -0.668                       | 5123         | -8968        | No effect                             |

**Figure G-1 Difference Between the Means ( $O_2$ ) and 95% CI for the Linear Combination of 25 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol**

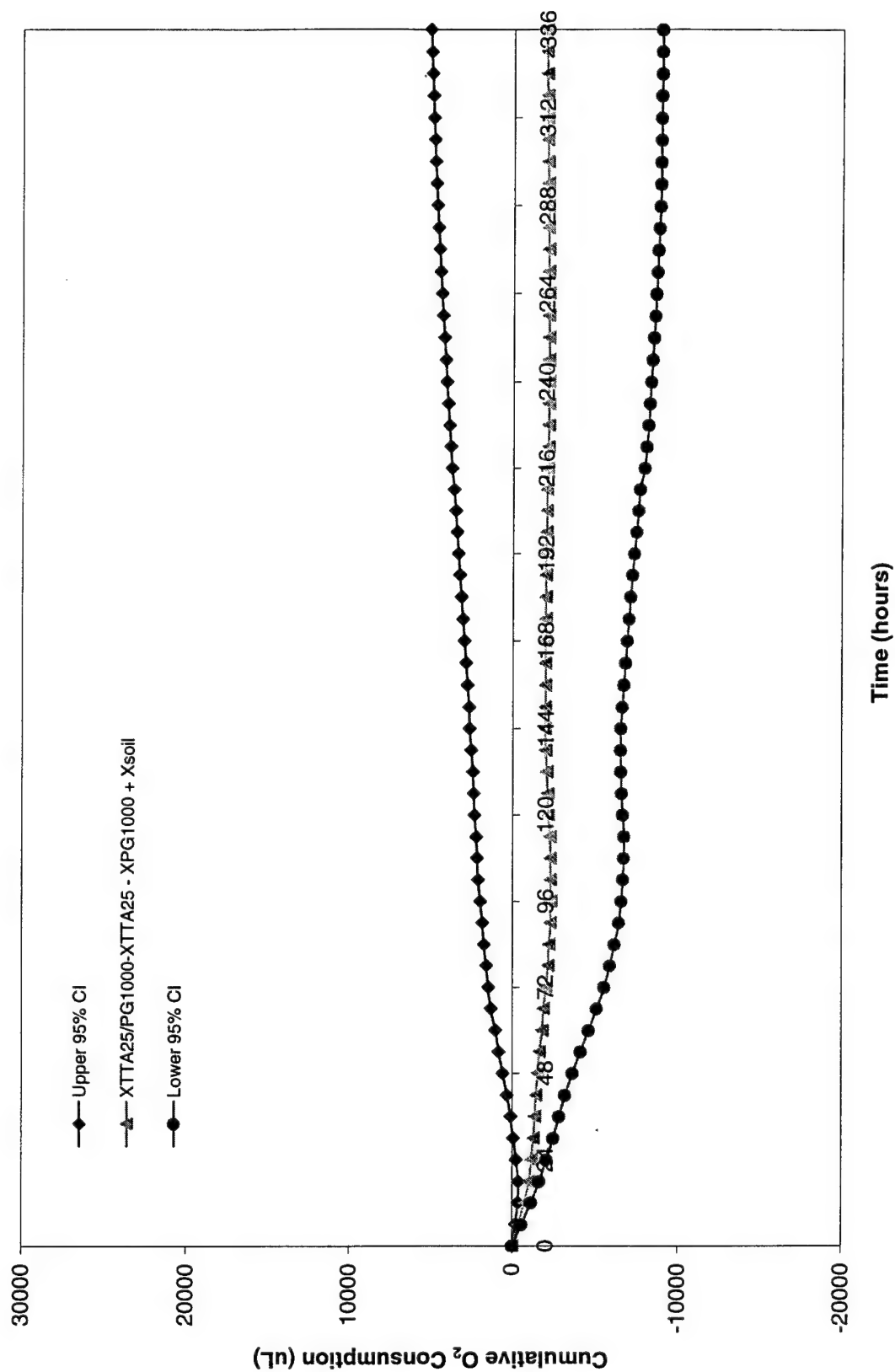


Table G-2 (Run-2) Data (O<sub>2</sub>) for Determining Biodegradation of 250 mg/kg Tolytriazole and 1,000 mg/kg Propylene Glycol

| Time (hours) | Mean Blank Soil | Std Dev Blank Soil | Mean TTA250 | Std Dev TTA250 | Mean PG1000 | Std Dev PG1000 | Mean TTA250 | Std Dev TTA250 | Mean PG1000 | Std Dev PG1000 | Pooled Estimator | Std Error | $\frac{X_{TTA250} - X_{PG1000}}{100 \times X_{TTA250} + X_{PG1000}}$ | Calc T Value (Tcrit = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /Inhibition/ No effect |
|--------------|-----------------|--------------------|-------------|----------------|-------------|----------------|-------------|----------------|-------------|----------------|------------------|-----------|--|------------------------------|--------------|--------------|---------------------------------------|
| 0            | 0               | 0                  | 0           | 0              | 0           | 0              | 0           | 0              | 0           | 0              | 0                | 0         | 0  | 0.000                        | 0            | 0            | N/A                                   |
| 6            | 960             | 90                 | 907         | 80             | 2305        | 66             | 1712        | 56             | 4504        | 65             | 4504             | 65        | -540   | -8.328                       | -381         | -699         | Inhibition                            |
| 12           | 1502            | 146                | 1470        | 135            | 3927        | 150            | 2906        | 125            | 16778       | 125            | 16778            | 125       | -988   | -7.895                       | -682         | -1294        | Inhibition                            |
| 18           | 1894            | 201                | 1877        | 185            | 5793        | 260            | 4026        | 199            | 40347       | 194            | 40347            | 194       | -1750  | -9.016                       | -1275        | -2225        | Inhibition                            |
| 24           | 2180            | 243                | 2188        | 225            | 8268        | 423            | 5155        | 276            | 83894       | 280            | 83894            | 280       | -3121  | -11.155                      | -2437        | -3806        | Inhibition                            |
| 30           | 2414            | 268                | 2453        | 258            | 11042       | 588            | 6351        | 359            | 144347      | 367            | 144347           | 367       | -4729  | -12.885                      | -3831        | -5628        | Inhibition                            |
| 36           | 2649            | 285                | 2726        | 291            | 13788       | 758            | 7742        | 458            | 227353      | 461            | 227353           | 461       | -6123  | -13.292                      | -4996        | -7250        | Inhibition                            |
| 42           | 2857            | 292                | 2993        | 322            | 16399       | 812            | 9335        | 525            | 270528      | 502            | 270528           | 502       | -7199  | -14.327                      | -5970        | -8429        | Inhibition                            |
| 48           | 3038            | 296                | 3232        | 352            | 18855       | 861            | 11116       | 584            | 312365      | 540            | 312365           | 540       | -7933  | -14.692                      | -6612        | -9254        | Inhibition                            |
| 54           | 3196            | 296                | 3424        | 377            | 21017       | 970            | 13036       | 676            | 395807      | 608            | 395807           | 608       | -8210  | -13.508                      | -6723        | -9698        | Inhibition                            |
| 60           | 3347            | 297                | 3642        | 403            | 23139       | 995            | 15330       | 784            | 452965      | 650            | 452965           | 650       | -8104  | -12.463                      | -6513        | -9695        | Inhibition                            |
| 66           | 3483            | 300                | 3818        | 425            | 25087       | 1014           | 17800       | 893            | 512822      | 692            | 512822           | 692       | -7622  | -11.017                      | -5929        | -9315        | Inhibition                            |
| 72           | 3632            | 298                | 4002        | 438            | 26824       | 1109           | 20396       | 1023           | 628023      | 766            | 628023           | 766       | -6798  | -8.880                       | -4925        | -8672        | Inhibition                            |
| 78           | 3817            | 294                | 4237        | 461            | 28328       | 1086           | 23037       | 1109           | 666236      | 789            | 666236           | 789       | -5711  | -7.242                       | -3781        | -7641        | Inhibition                            |
| 84           | 4006            | 294                | 4468        | 482            | 29458       | 988            | 25646       | 1238           | 696023      | 806            | 696023           | 806       | -4274  | -5.302                       | -2301        | -6246        | Inhibition                            |
| 90           | 4198            | 291                | 4697        | 492            | 30248       | 919            | 28150       | 1347           | 736366      | 829            | 736366           | 829       | -2597  | -3.133                       | -569         | -4826        | Inhibition                            |
| 96           | 4382            | 285                | 4923        | 513            | 30848       | 839            | 30552       | 1460           | 784919      | 856            | 784919           | 856       | -837   | -0.977                       | 1258         | -2931        | Inhibition                            |
| 102          | 4557            | 279                | 5140        | 529            | 31305       | 803            | 32804       | 1533           | 828478      | 879            | 828478           | 879       | 916  | 1.042                        | 3068         | -1236        | Inhibition                            |
| 108          | 4700            | 273                | 5321        | 547            | 31644       | 806            | 34805       | 1582           | 872513      | 902            | 872513           | 902       | 2540   | 2.815                        | 4748         | 332          | Inhibition                            |
| 114          | 4843            | 269                | 5493        | 563            | 31930       | 820            | 36615       | 1609           | 903681      | 918            | 903681           | 918       | 4034   | 4.393                        | 6282         | 1787         | Inhibition                            |
| 120          | 4963            | 258                | 5625        | 577            | 32177       | 832            | 38194       | 1625           | 924820      | 929            | 924820           | 929       | 5355   | 5.763                        | 7628         | 3081         | Inhibition                            |
| 126          | 5090            | 250                | 5780        | 589            | 32417       | 842            | 39489       | 1657           | 958003      | 946            | 958003           | 946       | 6381   | 6.748                        | 8695         | 4067         | Inhibition                            |
| 132          | 5210            | 244                | 5922        | 598            | 32645       | 840            | 40623       | 1684           | 982689      | 958            | 982689           | 958       | 7266   | 7.587                        | 9610         | 4923         | No effect                             |
| 138          | 5334            | 236                | 6066        | 610            | 32869       | 840            | 41549       | 1716           | 1012365     | 972            | 1012365          | 972       | 7948   | 8.177                        | 10327        | 5569         | No effect                             |
| 144          | 5448            | 230                | 6200        | 617            | 33058       | 845            | 42257       | 1729           | 1027290     | 979            | 1027290          | 979       | 8447   | 8.627                        | 10843        | 6051         | No effect                             |
| 150          | 5548            | 220                | 6311        | 630            | 33250       | 844            | 42859       | 1729           | 1031009     | 981            | 1031009          | 981       | 8846   | 9.018                        | 11247        | 6446         | Biodegradation                        |
| 156          | 5652            | 211                | 6439        | 635            | 33447       | 843            | 43413       | 1720           | 1023908     | 978            | 1023908          | 978       | 9179   | 9.390                        | 11571        | 6787         | Biodegradation                        |
| 162          | 5762            | 202                | 6551        | 642            | 33644       | 841            | 43885       | 1719           | 1023777     | 978            | 1023777          | 978       | 9453   | 9.670                        | 11844        | 7061         | Biodegradation                        |
| 168          | 5875            | 192                | 6682        | 647            | 33833       | 839            | 44309       | 1716           | 1021743     | 977            | 1021743          | 977       | 9668   | 9.900                        | 12058        | 7278         | Biodegradation                        |
| 174          | 5960            | 185                | 6789        | 655            | 33984       | 838            | 44627       | 1719           | 1026043     | 979            | 1026043          | 979       | 9814   | 10.028                       | 12208        | 7419         | Biodegradation                        |
| 180          | 6038            | 176                | 6882        | 659            | 34131       | 841            | 44917       | 1719           | 1028119     | 980            | 1028119          | 980       | 9942   | 10.149                       | 12339        | 7545         | Biodegradation                        |
| 186          | 6117            | 163                | 6973        | 665            | 34271       | 844            | 45183       | 1727           | 1037828     | 984            | 1037828          | 984       | 10056  | 10.217                       | 12464        | 7648         | Biodegradation                        |
| 192          | 6188            | 155                | 7055        | 666            | 34402       | 840            | 45431       | 1733           | 1041320     | 986            | 1041320          | 986       | 10162  | 10.308                       | 12574        | 7749         | Biodegradation                        |
| 198          | 6251            | 148                | 7141        | 675            | 34525       | 839            | 45667       | 1739           | 1048450     | 989            | 1048450          | 989       | 10252  | 10.364                       | 12673        | 7832         | Biodegradation                        |
| 204          | 6324            | 140                | 7221        | 681            | 34655       | 836            | 45895       | 1749           | 1058176     | 994            | 1058176          | 994       | 10342  | 10.407                       | 12774        | 7911         | Biodegradation                        |
| 210          | 6389            | 131                | 7300        | 691            | 34782       | 834            | 46104       | 1756           | 1066687     | 998            | 1066687          | 998       | 10412  | 10.435                       | 12853        | 7970         | Biodegradation                        |
| 216          | 6459            | 124                | 7370        | 696            | 34903       | 835            | 46306       | 1767           | 1077754     | 1003           | 1077754          | 1003      | 10492  | 10.462                       | 12947        | 8038         | Biodegradation                        |
| 222          | 6532            | 116                | 7450        | 703            | 35028       | 832            | 46515       | 1773           | 1084184     | 1006           | 1084184          | 1006      | 10569  | 10.507                       | 13031        | 8108         | Biodegradation                        |
| 228          | 6601            | 110                | 7531        | 711            | 35148       | 831            | 46717       | 1780           | 1092477     | 1010           | 1092477          | 1010      | 10639  | 10.536                       | 13109        | 8168         | Biodegradation                        |
| 234          | 6669            | 105                | 7608        | 719            | 35265       | 831            | 46913       | 1786           | 1100491     | 1013           | 1100491          | 1013      | 10710  | 10.568                       | 13190        | 8230         | Biodegradation                        |



Table G-2 (Run-2) Data (O<sub>2</sub>) for Determining Biodegradation of 250 mg/kg Tolytriazole and 1,000 mg/kg Propylene Glycol

| Time (hours) | Mean Blank Soil | Std Dev Blank Soil | Mean TTA250 | Std Dev TTA250 | Mean PG1000 | Std Dev PG1000 | Mean PG1000 TTA250 | Std Dev PG1000 TTA250 | Pooled Estimator | Std Error | $\lambda_{TTA250PG1000} \cdot X_{TTA250} - X_{PG1000} + X_{soil}$ | Calc T Value (Tcrit = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /Inhibition/ No effect |
|--------------|-----------------|--------------------|-------------|----------------|-------------|----------------|--------------------|-----------------------|------------------|-----------|---|------------------------------|--------------|--------------|---------------------------------------|
| 240          | 6736            | 97                 | 7688        | 728            | 35380       | 829            | 47105              | 1792                  | 1108312          | 1017      | 10774   | 10.593                       | 13262        | 8285         | Biodegradation                        |
| 246          | 6800            | 90                 | 7758        | 735            | 35494       | 829            | 47286              | 1798                  | 1116418          | 1021      | 10834   | 10.613                       | 13332        | 8336         | Biodegradation                        |
| 252          | 6864            | 84                 | 7832        | 743            | 35604       | 832            | 47467              | 1807                  | 1127960          | 1026      | 10895   | 10.619                       | 13406        | 8384         | Biodegradation                        |
| 258          | 6921            | 81                 | 7895        | 751            | 35704       | 830            | 47632              | 1812                  | 1134938          | 1029      | 10953   | 10.642                       | 13471        | 8434         | Biodegradation                        |
| 264          | 6987            | 78                 | 7964        | 758            | 35810       | 828            | 47799              | 1820                  | 1143847          | 1033      | 11012   | 10.658                       | 13540        | 8483         | Biodegradation                        |
| 270          | 7053            | 74                 | 8036        | 766            | 35913       | 830            | 47965              | 1825                  | 1152600          | 1037      | 11069   | 10.672                       | 13607        | 8531         | Biodegradation                        |
| 276          | 7114            | 73                 | 8108        | 770            | 36012       | 830            | 48123              | 1832                  | 1160619          | 1041      | 11117   | 10.681                       | 13664        | 8570         | Biodegradation                        |
| 282          | 7176            | 65                 | 8175        | 777            | 36112       | 832            | 48282              | 1838                  | 1169573          | 1045      | 11171   | 10.692                       | 13727        | 8614         | Biodegradation                        |
| 288          | 7232            | 61                 | 8236        | 786            | 36205       | 834            | 48431              | 1846                  | 1180916          | 1050      | 11222   | 10.689                       | 13791        | 8653         | Biodegradation                        |
| 294          | 7284            | 56                 | 8296        | 795            | 36295       | 833            | 48588              | 1852                  | 1188901          | 1053      | 11262   | 10.691                       | 13839        | 8684         | Biodegradation                        |
| 300          | 7336            | 53                 | 8350        | 802            | 36380       | 833            | 48705              | 1858                  | 1197402          | 1057      | 11311   | 10.700                       | 13898        | 8724         | Biodegradation                        |
| 306          | 7382            | 49                 | 8398        | 811            | 36459       | 835            | 48835              | 1865                  | 1208573          | 1062      | 11360   | 10.696                       | 13958        | 8761         | Biodegradation                        |
| 312          | 7439            | 44                 | 8462        | 817            | 36546       | 836            | 48977              | 1871                  | 1217230          | 1066      | 11406   | 10.701                       | 14014        | 8798         | Biodegradation                        |
| 318          | 7494            | 43                 | 8525        | 823            | 36629       | 842            | 49113              | 1879                  | 1228885          | 1071      | 11454   | 10.695                       | 14074        | 8833         | Biodegradation                        |
| 324          | 7549            | 47                 | 8597        | 829            | 36712       | 844            | 49254              | 1884                  | 1238015          | 1075      | 11493   | 10.692                       | 14124        | 8863         | Biodegradation                        |
| 330          | 7604            | 49                 | 8658        | 837            | 36790       | 848            | 49387              | 1891                  | 1249281          | 1080      | 11543   | 10.690                       | 14185        | 8901         | Biodegradation                        |
| 336          | 7654            | 53                 | 8719        | 843            | 36870       | 847            | 49517              | 1898                  | 1257653          | 1083      | 11582   | 10.691                       | 14234        | 8931         | Biodegradation                        |

**Figure G-2 Difference Between the Means ( $O_2$ ) and 95% CI for the Linear Combination of 250 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol**

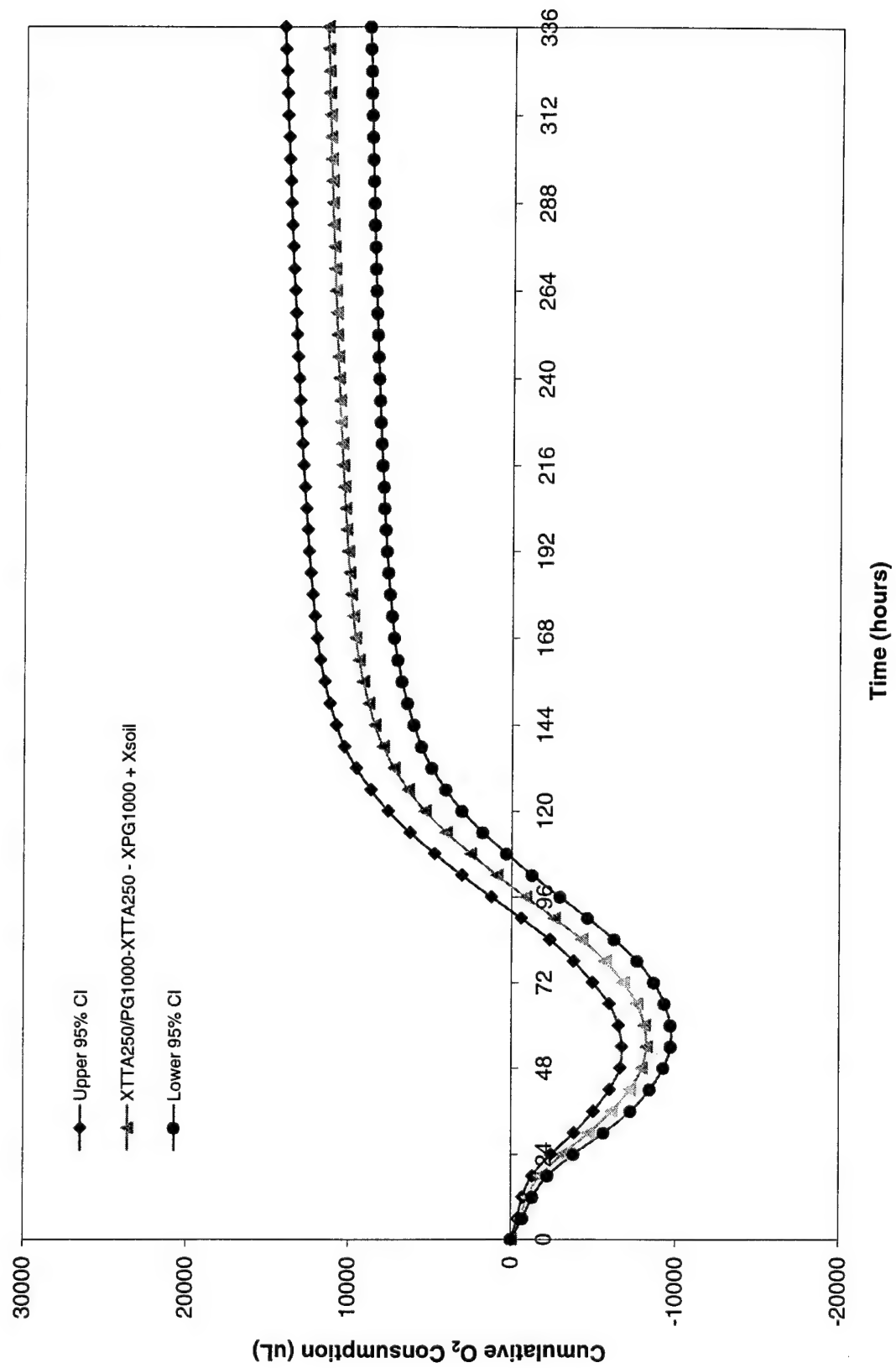


Table G-3 (Run-3) Data (O<sub>2</sub>) for Determining Biodegradation of 500 mg/kg Tolyltriazole and 1,000 mg/kg Propylene Glycol

| Time (hours) | Mean Blank Soil | Std Dev Blank Soil | Mean TTA500 | Std Dev TTA500 | Mean PG1000 | Std Dev PG1000 | Mean TTA500 | Std Dev TTA500 | Mean PG1000 | Std Dev PG1000 | Pooled Estimator | Std Error | $X_{TTA500PG1000} - X_{PG1000} + X_{oil}$ | Calc T Value (Tcrit = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /Inhibition/ No effect |
|--------------|-----------------|--------------------|-------------|----------------|-------------|----------------|-------------|----------------|-------------|----------------|------------------|-----------|---|------------------------------|--------------|--------------|---------------------------------------|
| 0            | 0               | 0                  | 0           | 0              | 0           | 0              | 0           | 0              | 0           | 0              | 0                | 0         | 0   | 0.000                        | 0            | 0            | N/A                                   |
| 6            | 610             | 25                 | 683         | 37             | 2189        | 61             | 1188        | 20             | 1462        | 20             | 1462             | 37        | -1074                                     | -29.079                      | -984         | -1165        | Inhibition                            |
| 12           | 981             | 29                 | 1147        | 84             | 3944        | 124            | 2078        | 28             | 5945        | 28             | 5945             | 74        | -2032                                     | -27.275                      | -1849        | -2214        | Inhibition                            |
| 18           | 1258            | 34                 | 1563        | 123            | 6240        | 233            | 2941        | 49             | 18087       | 49             | 18087            | 130       | -3604                                     | -27.740                      | -3286        | -3922        | Inhibition                            |
| 24           | 1493            | 36                 | 1940        | 158            | 8994        | 354            | 3831        | 67             | 38788       | 67             | 38788            | 190       | -5610                                     | -29.485                      | -5144        | -6076        | Inhibition                            |
| 30           | 1725            | 43                 | 2269        | 189            | 11593       | 452            | 4763        | 92             | 62370       | 92             | 62370            | 241       | -7374                                     | -30.563                      | -6784        | -7964        | Inhibition                            |
| 36           | 1949            | 48                 | 2582        | 220            | 14043       | 539            | 5762        | 121            | 88745       | 121            | 88745            | 288       | -8915                                     | -30.976                      | -8211        | -9619        | Inhibition                            |
| 42           | 2169            | 35                 | 2887        | 247            | 16312       | 568            | 6816        | 140            | 101086      | 140            | 101086           | 307       | -10214                                    | -33.253                      | -9462        | -10966       | Inhibition                            |
| 48           | 2361            | 34                 | 3161        | 271            | 18520       | 622            | 7930        | 174            | 122636      | 174            | 122636           | 338       | -11389                                    | -33.664                      | -10561       | -12217       | Inhibition                            |
| 54           | 2544            | 40                 | 3394        | 295            | 20489       | 638            | 9066        | 204            | 133878      | 204            | 133878           | 353       | -12273                                    | -34.719                      | -11408       | -13138       | Inhibition                            |
| 60           | 2718            | 40                 | 3631        | 316            | 22295       | 624            | 10301       | 194            | 131958      | 194            | 131958           | 351       | -12907                                    | -36.779                      | -12048       | -13766       | Inhibition                            |
| 66           | 2878            | 48                 | 3826        | 337            | 23960       | 630            | 11633       | 273            | 146671      | 273            | 146671           | 370       | -13274                                    | -35.877                      | -12369       | -14180       | Inhibition                            |
| 72           | 3032            | 60                 | 4053        | 359            | 25442       | 630            | 13085       | 370            | 165981      | 370            | 165981           | 394       | -13378                                    | -33.988                      | -12415       | -14341       | Inhibition                            |
| 78           | 3197            | 70                 | 4232        | 372            | 26650       | 571            | 14591       | 493            | 177467      | 493            | 177467           | 407       | -13094                                    | -32.173                      | -12098       | -14090       | Inhibition                            |
| 84           | 3357            | 79                 | 4417        | 391            | 27646       | 513            | 16209       | 637            | 206245      | 637            | 206245           | 439       | -12498                                    | -28.486                      | -11424       | -13571       | Inhibition                            |
| 90           | 3519            | 92                 | 4590        | 409            | 28411       | 456            | 17860       | 794            | 252425      | 794            | 252425           | 485       | -11821                                    | -23.942                      | -10433       | -12809       | Inhibition                            |
| 96           | 3673            | 113                | 4779        | 424            | 28994       | 421            | 19597       | 958            | 320591      | 958            | 320591           | 547       | -10503                                    | -19.200                      | -9164        | -11841       | Inhibition                            |
| 102          | 3823            | 123                | 4957        | 442            | 29441       | 398            | 21351       | 1135           | 412756      | 1135           | 412756           | 621       | -9224                                     | -14.861                      | -7705        | -10743       | Inhibition                            |
| 108          | 3968            | 134                | 5117        | 457            | 29819       | 407            | 23134       | 1322           | 532435      | 1322           | 532435           | 705       | -7834                                     | -11.113                      | -6109        | -9559        | Inhibition                            |
| 114          | 4060            | 140                | 5210        | 467            | 30113       | 415            | 24870       | 1488           | 653730      | 1488           | 653730           | 781       | -6393                                     | -8.185                       | -4482        | -8305        | Inhibition                            |
| 120          | 4231            | 153                | 5428        | 485            | 30413       | 414            | 26718       | 1658           | 791772      | 1658           | 791772           | 860       | -4892                                     | -5.690                       | -2788        | -6995        | Inhibition                            |
| 126          | 4387            | 165                | 5630        | 498            | 30696       | 431            | 28678       | 1845           | 963231      | 1845           | 963231           | 948       | -3260                                     | -3.439                       | -940         | -5581        | Inhibition                            |
| 132          | 4566            | 177                | 5850        | 514            | 30983       | 444            | 30698       | 2002           | 1120843     | 2002           | 1120843          | 1023      | -1570                                     | -1.535                       | 933          | -4072        | no effect                             |
| 138          | 4730            | 188                | 6053        | 536            | 31253       | 445            | 32582       | 2127           | 1257074     | 2127           | 1257074          | 1083      | 5   | 0.005                        | 2656         | -2645        | no effect                             |
| 144          | 4888            | 204                | 6256        | 549            | 31524       | 460            | 34406       | 2211           | 1356005     | 2211           | 1356005          | 1125      | 1514                                      | 1.346                        | 4267         | -1238        | no effect                             |
| 150          | 5068            | 219                | 6468        | 568            | 31806       | 470            | 36153       | 2266           | 1425743     | 2266           | 1425743          | 1154      | 2947                                      | 2.555                        | 5770         | 124          | Biodegradation                        |
| 156          | 5226            | 229                | 6641        | 583            | 32059       | 466            | 37776       | 2284           | 1449373     | 2284           | 1449373          | 1163      | 4302                                      | 3.699                        | 7148         | 1456         | Biodegradation                        |
| 162          | 5388            | 248                | 6822        | 599            | 32318       | 477            | 39284       | 2266           | 1438670     | 2266           | 1438670          | 1159      | 5533                                      | 4.775                        | 8369         | 2697         | Biodegradation                        |
| 168          | 5532            | 262                | 6996        | 615            | 32553       | 478            | 40601       | 2261           | 1438441     | 2261           | 1438441          | 1159      | 6585                                      | 5.683                        | 9420         | 3750         | Biodegradation                        |
| 174          | 5677            | 280                | 7150        | 630            | 32777       | 475            | 41825       | 2222           | 1399470     | 2222           | 1399470          | 1143      | 7575                                      | 6.628                        | 10371        | 4778         | Biodegradation                        |
| 180          | 5813            | 290                | 7290        | 641            | 32992       | 482            | 42915       | 2177           | 1356271     | 2177           | 1356271          | 1125      | 8445                                      | 7.506                        | 11199        | 5692         | Biodegradation                        |
| 186          | 5958            | 301                | 7448        | 654            | 33204       | 489            | 43872       | 2140           | 1323485     | 2140           | 1323485          | 1111      | 9178                                      | 8.258                        | 11898        | 6459         | Biodegradation                        |
| 192          | 6081            | 308                | 7572        | 667            | 33389       | 487            | 44714       | 2089           | 1272981     | 2089           | 1272981          | 1090      | 9833                                      | 9.021                        | 12501        | 7166         | Biodegradation                        |
| 198          | 6208            | 317                | 7704        | 679            | 33576       | 493            | 45451       | 2050           | 1239562     | 2050           | 1239562          | 1076      | 10379                                     | 9.649                        | 13011        | 7747         | Biodegradation                        |
| 204          | 6323            | 328                | 7817        | 694            | 33754       | 497            | 46116       | 1996           | 1191196     | 1996           | 1191196          | 1054      | 10868                                     | 10.307                       | 13448        | 8288         | Biodegradation                        |
| 210          | 6436            | 339                | 7929        | 705            | 33919       | 495            | 46694       | 1948           | 1148786     | 1948           | 1148786          | 1035      | 11282                                     | 10.896                       | 13816        | 8748         | Biodegradation                        |
| 216          | 6567            | 348                | 8081        | 717            | 34093       | 501            | 47248       | 1901           | 1109695     | 1901           | 1109695          | 1018      | 11640                                     | 11.437                       | 14130        | 9149         | Biodegradation                        |
| 222          | 6693            | 355                | 8213        | 724            | 34261       | 501            | 47744       | 1849           | 1064429     | 1849           | 1064429          | 997       | 11964                                     | 12.003                       | 14403        | 9525         | Biodegradation                        |
| 228          | 6805            | 362                | 8329        | 733            | 34414       | 498            | 48173       | 1813           | 1034619     | 1813           | 1034619          | 983       | 12234                                     | 12.450                       | 14839        | 9830         | Biodegradation                        |
| 234          | 6917            | 371                | 8438        | 745            | 34570       | 500            | 48567       | 1776           | 1007282     | 1776           | 1007282          | 970       | 12476                                     | 12.867                       | 14849        | 10104        | Biodegradation                        |

Table G-3 (Run-3) Data (O<sub>2</sub>) for Determining Biodegradation of 500 mg/kg Tolytriazole and 1,000 mg/kg Propylene Glycol

| Time (hours) | Mean Blank Soil | Std Dev Blank Soil | Mean TTA500 | Std Dev TTA500 | Mean PG1000 | Std Dev PG1000 | Mean PG1000 TTA500 | Std Dev PG1000 TTA500 | Pooled Estimator | Std Error | $\lambda_{TTA500PG1000} \cdot X_{TTA500} - X_{PG1000} + X_{cell}$ | Calc T Value (Tcrit = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /inhibition/ No effect |
|--------------|-----------------|--------------------|-------------|----------------|-------------|----------------|--------------------|-----------------------|------------------|-----------|---|------------------------------|--------------|--------------|---------------------------------------|
| 240          | 7022            | 383                | 8570        | 758            | 34717       | 502            | 48938              | 1738                  | 980021           | 956       | 12673   | 13.251                       | 15014        | 10333        | Biodegradation                        |
| 246          | 7143            | 390                | 8679        | 772            | 34867       | 501            | 49286              | 1714                  | 964946           | 949       | 12883   | 13.575                       | 15205        | 10561        | Biodegradation                        |
| 252          | 7256            | 397                | 8806        | 779            | 35012       | 502            | 49610              | 1690                  | 948931           | 941       | 13048   | 13.864                       | 15351        | 10745        | Biodegradation                        |
| 258          | 7362            | 405                | 8923        | 792            | 35154       | 505            | 49905              | 1673                  | 941021           | 937       | 13191   | 14.076                       | 15484        | 10898        | Biodegradation                        |
| 264          | 7482            | 410                | 9065        | 802            | 35290       | 501            | 50203              | 1662                  | 934917           | 934       | 13331   | 14.271                       | 15616        | 11045        | Biodegradation                        |
| 270          | 7593            | 422                | 9189        | 812            | 35428       | 504            | 50477              | 1655                  | 935146           | 934       | 13453   | 14.400                       | 15739        | 11167        | Biodegradation                        |
| 276          | 7688            | 432                | 9294        | 823            | 35554       | 505            | 50722              | 1653                  | 939296           | 936       | 13562   | 14.484                       | 15853        | 11271        | Biodegradation                        |
| 282          | 7782            | 438                | 9379        | 833            | 35678       | 504            | 50945              | 1653                  | 944550           | 939       | 13670   | 14.560                       | 15968        | 11373        | Biodegradation                        |
| 288          | 7881            | 448                | 9497        | 845            | 35803       | 505            | 51176              | 1653                  | 950329           | 942       | 13758   | 14.608                       | 16062        | 11453        | Biodegradation                        |
| 294          | 7980            | 454                | 9612        | 855            | 35926       | 507            | 51396              | 1660                  | 961380           | 947       | 13838   | 14.608                       | 16156        | 11520        | Biodegradation                        |
| 300          | 8081            | 462                | 9725        | 862            | 36050       | 507            | 51619              | 1665                  | 969652           | 951       | 13925   | 14.638                       | 16253        | 11597        | Biodegradation                        |
| 306          | 8180            | 470                | 9837        | 869            | 36171       | 511            | 51832              | 1671                  | 979557           | 956       | 14003   | 14.645                       | 16343        | 11664        | Biodegradation                        |
| 312          | 8277            | 482                | 9949        | 882            | 36296       | 514            | 52041              | 1681                  | 996554           | 964       | 14073   | 14.592                       | 16433        | 11713        | Biodegradation                        |
| 318          | 8382            | 493                | 10065       | 889            | 36419       | 513            | 52248              | 1689                  | 1006670          | 969       | 14146   | 14.594                       | 16518        | 11774        | Biodegradation                        |
| 324          | 8476            | 500                | 10172       | 899            | 36530       | 518            | 52441              | 1694                  | 1017801          | 975       | 14215   | 14.584                       | 16600        | 11830        | Biodegradation                        |
| 330          | 8549            | 511                | 10279       | 906            | 36630       | 522            | 52611              | 1707                  | 1034483          | 983       | 14251   | 14.503                       | 16855        | 11846        | Biodegradation                        |
| 336          | 8624            | 514                | 10380       | 912            | 36729       | 520            | 52776              | 1716                  | 1044643          | 987       | 14291   | 14.473                       | 16707        | 11875        | Biodegradation                        |

**Figure G-3 Difference Between the Means ( $O_2$ ) and 95% CI for the Linear Combination of 500 mg/kg Tolytriazole and 1,000 mg/kg Propylene Glycol**

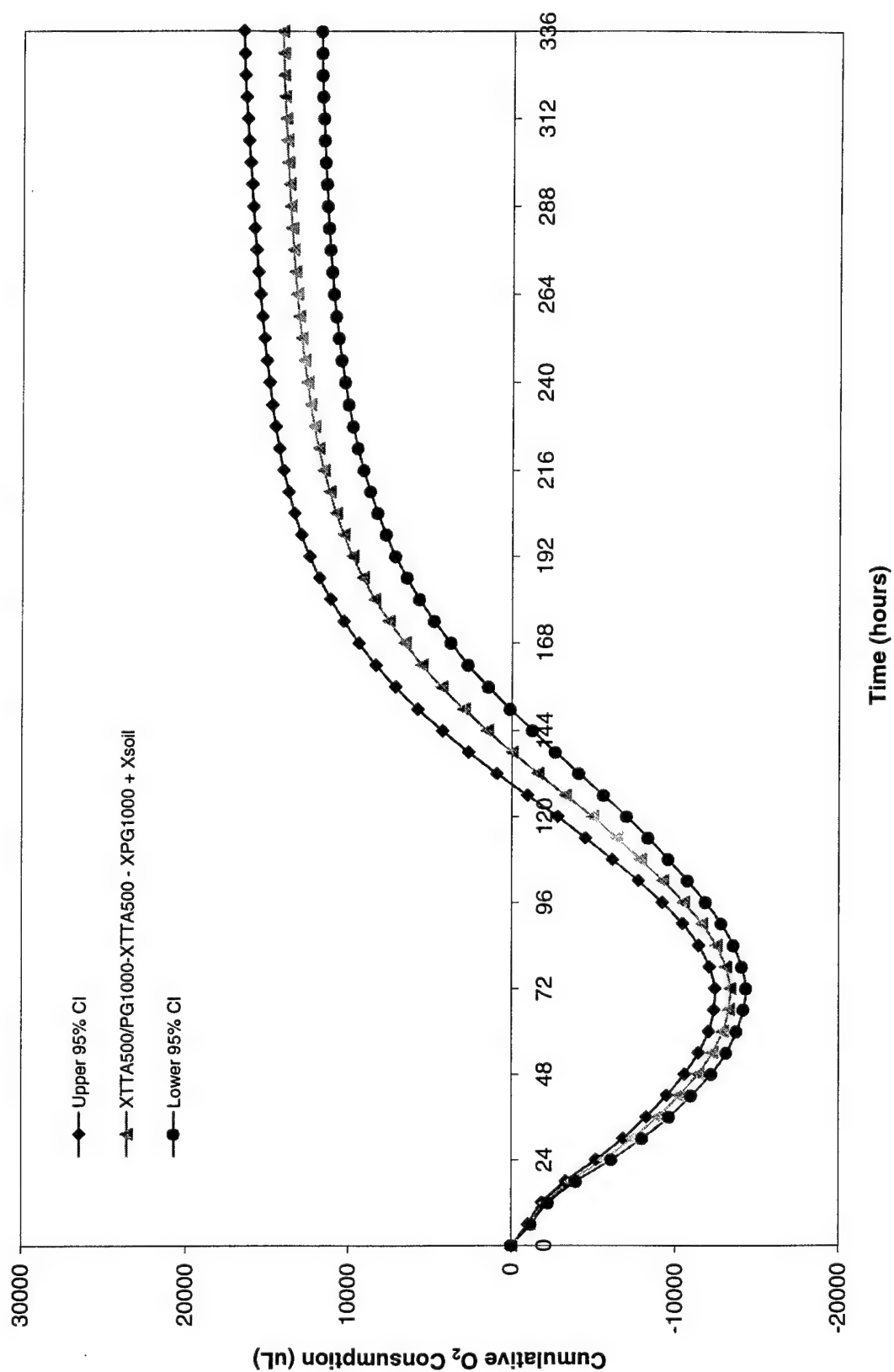


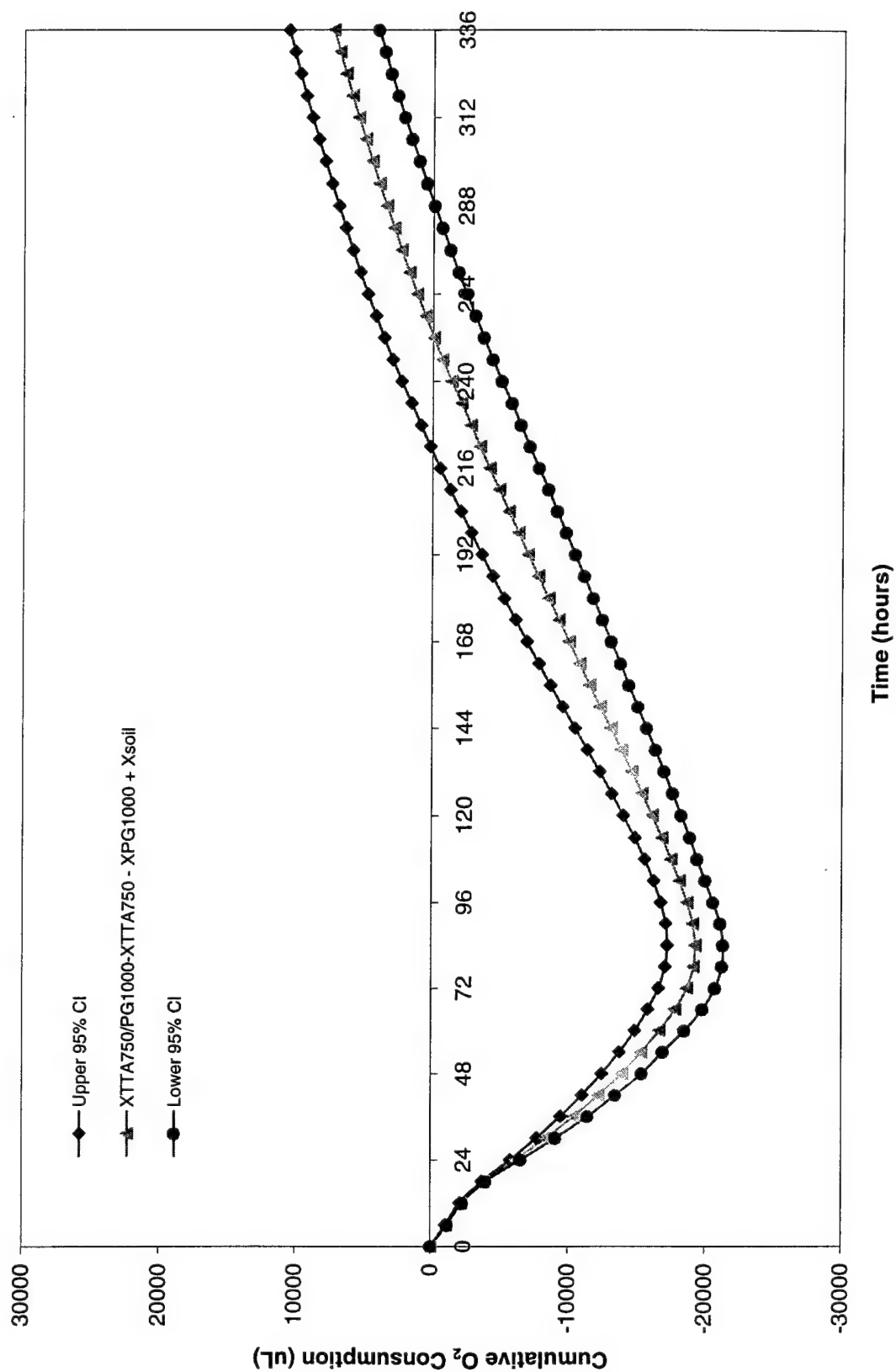
Table G-4 (Run-5) Data (O<sub>2</sub>) for Determining Biodegradation of 750 mg/kg Tolytriazole and 1,000 mg/kg Propylene Glycol

| Time<br>(hours) | (Run-3)<br>Mean<br>Blank<br>Soil | (Run-3)<br>Std Dev<br>Blank Soil | Mean<br>TTA750 | Std Dev<br>TTA750 | Mean<br>PG1000 | Std Dev<br>PG1000 | Mean<br>PG1000<br>TTA750 | Std Dev<br>PG1000<br>TTA750 | Pooled<br>Estimator | Std Error | $X_{TTA750PG1000} - X_{PG1000} + X_{soil}$ | Calc T Value<br>(Tcrit = 2.447) | Upper<br>95% CI | Lower<br>95% CI | Biodegradation<br>/Inhibition/<br>No effect |
|-----------------|----------------------------------|----------------------------------|----------------|-------------------|----------------|-------------------|--------------------------|-----------------------------|---------------------|-----------|--|---------------------------------|-----------------|-----------------|---|
| 0               | 0                                | 0                                | 0              | 0                 | 0              | 0                 | 0                        | 0                           | 0                   | 0         | 0.000                                      | 0                               | 0               | 0               | N/A   |
| 6               | 610                              | 25                               | 553            | 14                | 2161           | 53                | 948                      | 4                           | 843                 | 28        | -1156                                      | -41.222                         | -1088           | -1225           | Inhibition                                  |
| 12              | 981                              | 29                               | 950            | 24                | 3861           | 55                | 1615                     | 32                          | 1276                | 35        | -2215                                      | -64.179                         | -2131           | -2299           | Inhibition                                  |
| 18              | 1258                             | 34                               | 1306           | 44                | 6060           | 82                | 2224                     | 65                          | 3340                | 56        | -3864                                      | -69.571                         | -3748           | -4021           | Inhibition                                  |
| 24              | 1493                             | 36                               | 1608           | 60                | 8891           | 306               | 2800                     | 93                          | 26614               | 158       | -6206                                      | -39.377                         | -5820           | -6592           | Inhibition                                  |
| 30              | 1725                             | 43                               | 1913           | 77                | 11652          | 565               | 3418                     | 127                         | 85445               | 282       | -8242                                      | -29.822                         | -7731           | -9113           | Inhibition                                  |
| 36              | 1949                             | 48                               | 2212           | 93                | 14282          | 812               | 4080                     | 167                         | 174234              | 403       | -10464                                     | -25.949                         | -9477           | -11451          | Inhibition                                  |
| 42              | 2169                             | 35                               | 2505           | 106               | 16593          | 997               | 4782                     | 156                         | 257345              | 490       | -12247                                     | -24.989                         | -11048          | -13446          | Inhibition                                  |
| 48              | 2361                             | 34                               | 2796           | 110               | 19025          | 1216              | 5537                     | 196                         | 382317              | 597       | -13922                                     | -23.306                         | -12460          | -15383          | Inhibition                                  |
| 54              | 2544                             | 40                               | 3075           | 118               | 21152          | 1328              | 6346                     | 213                         | 455608              | 652       | -15337                                     | -23.519                         | -13741          | -16933          | Inhibition                                  |
| 60              | 2718                             | 40                               | 3351           | 124               | 23241          | 1514              | 7199                     | 247                         | 592736              | 744       | -16674                                     | -22.418                         | -14854          | -18494          | Inhibition                                  |
| 66              | 2878                             | 48                               | 3613           | 134               | 25171          | 1666              | 8088                     | 286                         | 719315              | 819       | -17818                                     | -21.746                         | -15813          | -19823          | Inhibition                                  |
| 72              | 3032                             | 60                               | 3864           | 140               | 26840          | 1712              | 9002                     | 347                         | 768038              | 847       | -18670                                     | -22.051                         | -16598          | -20741          | Inhibition                                  |
| 78              | 3197                             | 70                               | 4141           | 142               | 28208          | 1704              | 9980                     | 430                         | 777937              | 852       | -19173                                     | -22.500                         | -17087          | -21258          | Inhibition                                  |
| 84              | 3357                             | 79                               | 4395           | 150               | 29246          | 1653              | 10999                    | 506                         | 753096              | 838       | -19285                                     | -23.003                         | -17234          | -21337          | Inhibition                                  |
| 90              | 3519                             | 92                               | 4658           | 156               | 30018          | 1569              | 12044                    | 595                         | 711219              | 815       | -19113                                     | -23.459                         | -17119          | -21107          | Inhibition                                  |
| 96              | 3673                             | 113                              | 4877           | 158               | 30556          | 1452              | 13091                    | 694                         | 655358              | 782       | -18669                                     | -23.870                         | -16755          | -20583          | Inhibition                                  |
| 102             | 3823                             | 123                              | 5098           | 160               | 30981          | 1373              | 14138                    | 802                         | 640251              | 773       | -18118                                     | -23.438                         | -16227          | -20010          | Inhibition                                  |
| 108             | 3968                             | 134                              | 5329           | 170               | 31356          | 1342              | 15230                    | 916                         | 669375              | 790       | -17488                                     | -22.125                         | -15554          | -19422          | Inhibition                                  |
| 114             | 4060                             | 140                              | 5531           | 178               | 31685          | 1338              | 16301                    | 1034                        | 725183              | 823       | -16855                                     | -20.488                         | -14842          | -18668          | Inhibition                                  |
| 120             | 4231                             | 153                              | 5739           | 183               | 31985          | 1350              | 17384                    | 1161                        | 803378              | 866       | -16109                                     | -18.604                         | -13990          | -18228          | Inhibition                                  |
| 126             | 4387                             | 165                              | 5910           | 191               | 32264          | 1364              | 18418                    | 1288                        | 892279              | 913       | -15369                                     | -16.841                         | -13135          | -17602          | Inhibition                                  |
| 132             | 4566                             | 177                              | 6115           | 203               | 32542          | 1381              | 19485                    | 1418                        | 993476              | 963       | -14605                                     | -15.167                         | -12249          | -16962          | Inhibition                                  |
| 138             | 4730                             | 188                              | 6311           | 217               | 32809          | 1395              | 20535                    | 1548                        | 1101986             | 1014      | -13855                                     | -13.661                         | -11373          | -16336          | Inhibition                                  |
| 144             | 4888                             | 204                              | 6475           | 228               | 33048          | 1411              | 21559                    | 1675                        | 1217504             | 1066      | -13076                                     | -12.266                         | -10467          | -15684          | Inhibition                                  |
| 150             | 5068                             | 219                              | 6639           | 241               | 33290          | 1429              | 22568                    | 1797                        | 1337889             | 1117      | -12293                                     | -11.001                         | -9558           | -15027          | Inhibition                                  |
| 156             | 5226                             | 229                              | 6816           | 255               | 33534          | 1445              | 23602                    | 1914                        | 1460901             | 1168      | -11522                                     | -9.867                          | -8665           | -14379          | Inhibition                                  |
| 162             | 5388                             | 248                              | 7030           | 272               | 33793          | 1460              | 24646                    | 2024                        | 1582961             | 1215      | -10789                                     | -8.876                          | -7815           | -13764          | Inhibition                                  |
| 168             | 5532                             | 262                              | 7195           | 283               | 34008          | 1475              | 25653                    | 2117                        | 1693571             | 1257      | -10019                                     | -7.969                          | -6942           | -13095          | Inhibition                                  |
| 174             | 5677                             | 280                              | 7373           | 295               | 34237          | 1491              | 26656                    | 2204                        | 1801421             | 1297      | -9278                                      | -7.155                          | -6105           | -12451          | Inhibition                                  |
| 180             | 5813                             | 290                              | 7530           | 305               | 34451          | 1505              | 27644                    | 2284                        | 1904293             | 1333      | -8524                                      | -6.394                          | -5262           | -11787          | Inhibition                                  |
| 186             | 5958                             | 301                              | 7668           | 312               | 34650          | 1522              | 28587                    | 2354                        | 1999667             | 1366      | -7773                                      | -5.690                          | -4430           | -11116          | Inhibition                                  |
| 192             | 6081                             | 308                              | 7793           | 320               | 34829          | 1536              | 29509                    | 2416                        | 2086154             | 1395      | -7033                                      | -5.040                          | -3618           | -10447          | Inhibition                                  |
| 198             | 6208                             | 317                              | 7929           | 329               | 35013          | 1548              | 30419                    | 2470                        | 2164860             | 1421      | -6315                                      | -4.442                          | -2836           | -9793           | Inhibition                                  |
| 204             | 6323                             | 328                              | 8068           | 340               | 35194          | 1561              | 31398                    | 2516                        | 2234335             | 1444      | -5601                                      | -3.879                          | -2068           | -9135           | Inhibition                                  |
| 210             | 6436                             | 339                              | 8212           | 350               | 35381          | 1574              | 32256                    | 2553                        | 2293905             | 1463      | -4901                                      | -3.349                          | -1320           | -8481           | Inhibition                                  |
| 216             | 6567                             | 348                              | 8352           | 361               | 35562          | 1587              | 33171                    | 2583                        | 2344422             | 1479      | -4176                                      | -2.823                          | -557            | -7796           | Inhibition                                  |
| 222             | 6693                             | 355                              | 8493           | 372               | 35745          | 1601              | 34070                    | 2601                        | 2382887             | 1491      | -3476                                      | -2.331                          | 174             | -7125           | No effect                                   |
| 228             | 6805                             | 362                              | 8641           | 383               | 35924          | 1614              | 34963                    | 2612                        | 2410516             | 1500      | -2797                                      | -1.865                          | 873             | -6467           | No effect                                   |
| 234             | 6917                             | 371                              | 8761           | 393               | 36088          | 1629              | 35817                    | 2615                        | 2428717             | 1506      | -2114                                      | -1.404                          | 1570            | -5798           | No effect                                   |

Table G-4 (Run-5) Data (O<sub>2</sub>) for Determining Biodegradation of 750 mg/kg Tolytriazole and 1,000 mg/kg Propylene Glycol

| Time (hours) | (Run-3) Mean Blank Soil | (Run-3) Std Dev Blank Soil | Mean TTA750 | Std Dev TTA750 | Mean PG1000 | Std Dev PG1000 | Mean TTA750 | Std Dev TTA750 | Mean PG1000 | Std Dev PG1000 | Pooled Estimator | Std Error | $\lambda_{TTA750PG10} \times 10^3 \times \frac{X_{TTA750}}{X_{PG1000} + X_{cell}}$ | Calc T Value (Tcrit = 2.447) | Upper 95% CI | Lower 95% CI | Biodegradation /inhibition/ No effect |
|--------------|-------------------------|----------------------------|-------------|----------------|-------------|----------------|-------------|----------------|-------------|----------------|------------------|-----------|--|------------------------------|--------------|--------------|---------------------------------------|
| 240          | 7022                    | 383                        | 8752        | 393            | 36205       | 1648           | 36561       | 2603           | 36358       | 1663           | 2429474          | 1506      | -1374  | -0.912                       | 2311         | -5059        | No effect                             |
| 246          | 7143                    | 390                        | 8849        | 399            | 36358       | 1663           | 37344       | 2591           | 36358       | 1663           | 2428429          | 1506      | -720   | -0.478                       | 2964         | -4404        | No effect                             |
| 252          | 7256                    | 397                        | 8927        | 403            | 36496       | 1674           | 38108       | 2575           | 36496       | 1674           | 2419001          | 1503      | -59  | -0.040                       | 3617         | -3736        | No effect                             |
| 258          | 7362                    | 405                        | 9029        | 419            | 36636       | 1685           | 38861       | 2551           | 36636       | 1685           | 2401455          | 1497      | 558  | 0.372                        | 4221         | -3106        | No effect                             |
| 264          | 7482                    | 410                        | 9140        | 426            | 36771       | 1694           | 39606       | 2525           | 36771       | 1694           | 2377427          | 1490      | 1177   | 0.790                        | 4822         | -2468        | No effect                             |
| 270          | 7593                    | 422                        | 9257        | 435            | 36910       | 1705           | 40335       | 2495           | 36910       | 1705           | 2352995          | 1482      | 1761   | 1.188                        | 5387         | -1866        | No effect                             |
| 276          | 7688                    | 432                        | 9344        | 442            | 37023       | 1713           | 41030       | 2460           | 37023       | 1713           | 2318581          | 1471      | 2351   | 1.598                        | 5950         | -1249        | No effect                             |
| 282          | 7782                    | 438                        | 9452        | 449            | 37148       | 1720           | 41717       | 2423           | 37148       | 1720           | 2281777          | 1459      | 2899   | 1.986                        | 6470         | -672         | No effect                             |
| 288          | 7881                    | 448                        | 9537        | 455            | 37265       | 1729           | 42382       | 2382           | 37265       | 1729           | 2241816          | 1446      | 3461   | 2.393                        | 7001         | -79          | No effect                             |
| 294          | 7980                    | 454                        | 9635        | 461            | 37381       | 1735           | 43032       | 2342           | 37381       | 1735           | 2202705          | 1434      | 3995   | 2.786                        | 7504         | 487          | Biodegradation                        |
| 300          | 8081                    | 462                        | 9737        | 470            | 37494       | 1742           | 43675       | 2302           | 37494       | 1742           | 2164838          | 1421      | 4525   | 3.184                        | 8004         | 1047         | Biodegradation                        |
| 306          | 8180                    | 470                        | 9835        | 477            | 37595       | 1747           | 44285       | 2260           | 37595       | 1747           | 2124740          | 1408      | 5036   | 3.576                        | 8482         | 1590         | Biodegradation                        |
| 312          | 8277                    | 482                        | 9920        | 484            | 37702       | 1756           | 44890       | 2221           | 37702       | 1756           | 2091163          | 1397      | 5546   | 3.970                        | 8965         | 2127         | Biodegradation                        |
| 318          | 8382                    | 493                        | 10032       | 491            | 37819       | 1761           | 45497       | 2183           | 37819       | 1761           | 2057945          | 1386      | 6027   | 4.349                        | 9419         | 2636         | Biodegradation                        |
| 324          | 8476                    | 500                        | 10126       | 498            | 37925       | 1768           | 46079       | 2146           | 37925       | 1768           | 2025599          | 1375      | 6505   | 4.731                        | 9869         | 3140         | Biodegradation                        |
| 330          | 8549                    | 511                        | 10215       | 504            | 38020       | 1773           | 46631       | 2113           | 38020       | 1773           | 1998211          | 1366      | 6945   | 5.086                        | 10287        | 3603         | Biodegradation                        |
| 336          | 8624                    | 514                        | 10290       | 512            | 38109       | 1780           | 47169       | 2080           | 38109       | 1780           | 1972926          | 1357      | 7393   | 5.448                        | 10714        | 4073         | Biodegradation                        |

**Figure G-4 Difference Between the Means ( $O_2$ ) and 95% CI for the Linear Combination of 750 mg/kg Tolytriazole and 1,000 mg/kg Propylene Glycol**





## Appendix H: HPLC Analysis of Residual Tolyltriazole

HPLC analysis was performed on the amount of tolyltriazole residual available “before” and “after” respirometry analysis. HPLC analysis was performed for two concentration levels of tolyltriazole (25 – 250 mg/kg) within the soil. Tolyltriazole extracted/residuals had numerous pathways of degradation (biotic, absorption, chemical) before and after the respirometry experiments in soil.

Prior to analyses of tolyltriazole residuals in the soil environment, a calibration curve was established for HPLC analysis of tolyltriazole in a pure methanol (see Figure 3-2). Appendix C contains data and calculations. A subtle change in the specific gravity of extraction solutions occurred due to the additional H<sub>2</sub>O from the soil mixing with the methanol used for extracting tolyltriazole from soil. This change in the specific gravity was accounted for in the conversion of tolyltriazole residuals using the calibration curve equation.

A step-by-step process for determining the potential degradation/residual tolyltriazole in soil was performed on the soil treated with ADF components “before” respirometry runs, in Table H-1 through Table H-4. Table H-1 contains the data for determining the soils moisture.

Table H-1  
Calculations of Soil Moisture (Before Respirometry Experiments)

| Aluminum Container                      |                      |                                 |                                 |                     |                     |                             |                                  |
|---|----------------------|---------------------------------|---------------------------------|---------------------|---------------------|-----------------------------|----------------------------------|
|   | Wt of Alum Cont (gm) | Wt of Alum Cont & Wet Soil (gm) | Wt of Alum Cont & Dry Soil (gm) | Wt of Wet Soil (gm) | Wt of Dry Soil (gm) | Wt of H <sub>2</sub> O (gm) | % H <sub>2</sub> O in Spent Soil |
| TTA <sub>25</sub>                       | 1.5540               | 18.2035                         | 15.7225                         | 16.6495             | 14.1685             | 2.4810                      | 14.90%                           |
| TTA <sub>250</sub>                      | 1.5499               | 17.1490                         | 14.8478                         | 15.5991             | 13.2979             | 2.3012                      | 14.75%                           |
| TTA <sub>500</sub>                      | 1.5530               | 14.9002                         | 13.0375                         | 13.3472             | 11.4845             | 1.8627                      | 13.96%                           |
| PG <sub>1000</sub> & TTA <sub>25</sub>  | 1.5424               | 18.3540                         | 15.9437                         | 16.8116             | 14.4013             | 2.4103                      | 14.34%                           |
| PG <sub>1000</sub> & TTA <sub>250</sub> | 1.5462               | 18.8703                         | 16.2536                         | 17.3242             | 14.7074             | 2.6168                      | 15.10%                           |
| PG <sub>1000</sub> & TTA <sub>500</sub> | 1.5616               | 13.9762                         | 12.1161                         | 12.4146             | 10.5545             | 1.8601                      | 14.98%                           |

The measurements of soil moisture were determined through stand alone weight measurements of the soil media (see “Aluminum Container” section in the above data). A sample of the “wet” soil was weighed and measured, then dried at 85°C for 24 hrs, to obtain a “dry” soil sample. The weight of water removed from the soil was then calculated.

Table H-2 contains the weight measurements of the vials, methanol, and soil used in the HPLC analysis of “before” respirometry analysis (without biodegradation potential in soil). This was recalculated to determine the specific gravity of the mixture of methanol and H<sub>2</sub>O (from moisture content determined in Table H-1) used to extract the tolyltriazole.

**Table H-2**  
**Weights used in Removal Efficiency (Before Respirometry Experiments)**

| 40 mL EPA Vial for HPLC Extraction Methods |                          |                        |                                   |                                    |                                |
|--|--------------------------|------------------------|-----------------------------------|------------------------------------|--------------------------------|
|  | Wt of 40 mL Vial<br>(gm) | Wt Vial & Soil<br>(gm) | Wt Vial & Soil &<br>Methanol (gm) | Wt of Methanol in<br>the Vial (gm) | Wt of Soil in the<br>Vial (gm) |
| TTA <sub>25</sub>                          | 22.3196                  | 34.8771                | 46.2010                           | 11.3239                            | 12.5575                        |
| TTA <sub>250</sub>                         | 22.4522                  | 34.7269                | 46.2699                           | 11.5429                            | 12.2747                        |
| TTA <sub>500</sub>                         | 22.4064                  | 37.3024                | 48.8810                           | 11.5786                            | 14.8959                        |
| PG <sub>1000</sub> & TTA <sub>25</sub>     | 22.4317                  | 36.0177                | 47.6961                           | 11.6785                            | 13.5860                        |
| PG <sub>1000</sub> & TTA <sub>250</sub>    | 22.4771                  | 34.0338                | 45.5804                           | 11.5466                            | 11.5567                        |
| PG <sub>1000</sub> & TTA <sub>500</sub>    | 22.3717                  | 34.9987                | 46.6901                           | 11.6914                            | 12.6270                        |

**Note:** Upon inoculation and mixing of the soil with the chemical solution, immediate extraction was performed. This allowed the assumption of minimal biodegradation. The biodegradation was considered negligible since anaerobic conditions were introduced with the sealed vials and little oxygen due to the filled vial volume with aqueous solution. Photodegradation was assumed negligible by the use of amber color vials.

Table H-3 contains the HPLC detection area (mAu\*s) values for tolyltriazole residuals “before” respirometry (without biodegradation potential in soil).

**Table H-3**  
**HPLC Detection Areas for Tolyltriazole Residuals (Before Respirometry Experiments)**

| Soil Extracted Solution (Meth+ H <sub>2</sub> O + Toly) (mAu*s) |         |         |
|---|---------|---------|
|   | Average | Std Dev |
| TTA <sub>25</sub>   | 173.98  | 2.36    |
| TTA <sub>250</sub>  | 1522.90 | 5.50    |
| TTA <sub>500</sub>  | 3811.32 | 3.14    |
| PG <sub>1000</sub> & TTA <sub>25</sub>                          | 177.61  | 2.14    |
| PG <sub>1000</sub> & TTA <sub>250</sub>                         | 1519.16 | 2.72    |
| PG <sub>1000</sub> & TTA <sub>500</sub>                         | 3265.56 | 4.11    |

**Note:** Each HPLC detection area listed above represents three measurements averaged.

The preliminary information was now gathered on soil moisture, mass of vials/methanol/soil, and the detection areas associated with the “before” respirometry soil treatments. This allowed the calculations of residual tolyltriazole from interaction with soil shown in the following steps of calculations in Table H-4 shown below:

**Table H-4**  
**Steps/Calculations for the Recovery Percentage of Tolyltriazole Residuals**  
**(Before Respirometry Experiments)**

|   | 1                 | 2       | 3                 | 4   | 5   | 6            | 7               | 8               |                             |         |
|---|-------------------|---------|-------------------|---|---|--------------|-----------------|-----------------|-----------------------------|---------|
|   | HPLC Area (mAu*s) |         | Conc Conversion   | Density of Methanol/H <sub>2</sub> O mix in Vial                  | Mass of Toly in Vial                              | Soil in Vial | End Conc        | Initial Conc    | % recovered                 |         |
|   | Avg               | Std Dev | x = y/9.01 (mg/L) | (wtH <sub>2</sub> O+wtMeth) (volH <sub>2</sub> O+volMeth) (mg/mL) | [(conc/density)*(wt H2O + Meth)]/1000mL (mg toly) | (mg)         | mg toly kg soil | mg toly kg soil | (End Conc/Initial Conc)*100 |         |
|   |                   |         |                   |   |   |              |                 |                 | Avg                         | Std Dev |
| TTA <sub>25</sub>                       | 173.978           | 2.362   | 19.309            | 0.813   | 0.313   | 12.557       | 24.947          | 25.000          | 99.79%                      | 1.35%   |
| TTA <sub>250</sub>                      | 1522.904          | 5.504   | 169.024           | 0.812   | 2.779   | 12.275       | 226.389         | 250.000         | 90.56%                      | 0.33%   |
| TTA <sub>500</sub>                      | 3811.318          | 3.144   | 423.010           | 0.815   | 7.087   | 14.896       | 475.772         | 500.000         | 95.15%                      | 0.08%   |
| PG <sub>1000</sub> & TTA <sub>25</sub>  | 177.608           | 2.135   | 19.712            | 0.814   | 0.330   | 13.586       | 24.302          | 25.000          | 97.21%                      | 1.17%   |
| PG <sub>1000</sub> & TTA <sub>250</sub> | 1519.161          | 2.725   | 168.608           | 0.811   | 2.762   | 11.557       | 238.980         | 250.000         | 95.59%                      | 0.17%   |
| PG <sub>1000</sub> & TTA <sub>500</sub> | 3265.557          | 4.107   | 362.437           | 0.813   | 6.056   | 12.627       | 479.632         | 500.000         | 95.93%                      | 0.12%   |

**Step 1** The areas from HPLC analysis are listed in this step (y = areas). The equation (y = 9.01x) was derived in section 3.2 for the HPLC calibration curve for tolyltriazole.

**Step 2** Rearranging the equation to provide the measured concentration of tolyltriazole within the prepared 40-mL vial sample. The solution analyzed contains tolyltriazole + methanol + H<sub>2</sub>O from the soil, making the concentration slightly diluted.

**Step 3** The combined density of the methanol with H<sub>2</sub>O, volumes, and the mass of both solution types. Data reference is from the pre-measurements found in Table H-1

- Methanol mass is determined from pre-measurements
- H<sub>2</sub>O mass is determined from premeasurements (mass in vial \* moisture content of soil)
- Methanol volume is found from the known density (TTA = 0.786) divided by its mass
- H<sub>2</sub>O volume equals H<sub>2</sub>O mass

**Step 4** Using (step 3)\*(step 4)\*(Table H-1 data) / unit conversion (1L/1,000 mL)

**Step 5** The mass of soil in the vial (from Table H-1)

**Step 6** (step 4)/(step 5)\*unit conversion of soil (1 kg/1,000 mg)

**Step 7** Initial concentration of tolyltriazole in soil (mg chemical/kg soil)

**Step 8** [(step 6)/ (step 7)] \* 100%

The recovered tolyltriazole from interaction with the soil (without biodegradation potential) was now established for all of the possible chemical concentrations (shown above). The same procedures were followed for each of the different concentrations/residuals of tolyltriazole recovered "after" the respirometry experiments.

## Measurements of Tolyltriazole Residuals After Respirometry Experiments

Table H-5  
HPLC data for Tolyltriazole (25 mg/kg) Treatment of Uncontaminated Soil  
(After Respirometry Experiments)

| Treatment                              | Microcosm | Wt of 40 mL Vial (gm) | Wt Vial & Soil (gm) | Wt Vial & Soil & Methanol (gm) | Wt of Methanol in the Vial (gm) | Wt of Soil in the Vial (gm) |
|--|-----------|-----------------------|---------------------|--------------------------------|---------------------------------|-----------------------------|
| TTA <sub>25</sub>                      | 1         | 22.4438               | 29.3103             | 40.9506                        | 11.6403                         | 6.8665                      |
|  | 2         | 22.4796               | 30.0171             | 41.6036                        | 11.5865                         | 7.5375                      |
|  | 3         | 22.3229               | 29.5542             | 41.1647                        | 11.6105                         | 7.2313                      |
|  | 4         | 22.4637               | 29.7977             | 41.4635                        | 11.6659                         | 7.3339                      |
|  | 5         | 22.4028               | 30.3971             | 41.9554                        | 11.5583                         | 7.9943                      |
| PG <sub>1000</sub> & TTA <sub>25</sub> | 8         | 22.3728               | 29.2256             | 40.8275                        | 11.6019                         | 6.8528                      |
|  | 9         | 22.4878               | 30.6921             | 41.9831                        | 11.2910                         | 8.2043                      |
|  | 10        | 22.2239               | 29.7320             | 41.4520                        | 11.7199                         | 7.5081                      |
|  | 11        | 22.5094               | 30.3521             | 41.9602                        | 11.6081                         | 7.8426                      |
|  | 12        | 22.4167               | 30.8103             | 41.5259                        | 10.7156                         | 8.3936                      |

| Treatment                              | Microcosm | Wt of Alum Cont (gm) | Wt of Alum Cont & Wet Soil (gm) | Wt of Alum Cont & Dry Soil (gm) | Wt of Wet Soil (gm) | Wt of Dry Soil (gm) | Wt of H <sub>2</sub> O (gm) | % H <sub>2</sub> O in Spent Soil |
|--|-----------|----------------------|---------------------------------|---------------------------------|---------------------|---------------------|-----------------------------|----------------------------------|
| TTA <sub>25</sub>                      | 1         | 1.5590               | 16.3870                         | 15.1052                         | 14.8280             | 13.5462             | 1.2818                      | 8.64%                            |
|  | 2         | 1.5597               | 18.6702                         | 17.3878                         | 17.1105             | 15.8280             | 1.2825                      | 7.50%                            |
|  | 3         | 1.5587               | 17.0204                         | 15.8300                         | 15.4617             | 14.2713             | 1.1904                      | 7.70%                            |
|  | 4         | 1.5433               | 19.5058                         | 17.9913                         | 17.9625             | 16.4480             | 1.5145                      | 8.43%                            |
|  | 5         | 1.5404               | 17.6691                         | 16.4280                         | 16.1287             | 14.8876             | 1.2411                      | 7.69%                            |
| PG <sub>1000</sub> & TTA <sub>25</sub> | 8         | 1.5480               | 15.2804                         | 14.1787                         | 13.7324             | 12.6308             | 1.1017                      | 8.02%                            |
|  | 9         | 1.5541               | 17.5317                         | 16.0666                         | 15.9776             | 14.5125             | 1.4651                      | 9.17%                            |
|  | 10        | 1.5578               | 17.8573                         | 16.5190                         | 16.2995             | 14.9612             | 1.3383                      | 8.21%                            |
|  | 11        | 1.5476               | 18.5609                         | 17.0613                         | 17.0133             | 15.5138             | 1.4995                      | 8.81%                            |
|  | 12        | 1.5559               | 20.0553                         | 18.3610                         | 18.4994             | 16.8051             | 1.6943                      | 9.16%                            |

| Treatment                              | Microcosms | Average HPLC Area (mAu*s) | Conc.             | Density of Methanol/H <sub>2</sub> O mix in Vial                  | Mass of Toly in Vial   | Soil in Vial | End Conc        | Initial Conc    | % recovered                 |
|--|------------|---------------------------|-------------------|---|--|--------------|-----------------|-----------------|-----------------------------|
|  |            | y = 9.01x                 | x = y/9.01 (mg/L) | (wtH <sub>2</sub> O+wtMeth)/(volH <sub>2</sub> O+volMeth) (mg/mL) | [(conc/density)*(wt H <sub>2</sub> O + Meth)]/1000mL (mg toly) | (mg)         | mg toly kg soil | mg toly kg soil | (End Conc/Initial Conc)*100 |
| TTA <sub>25</sub>                      | 1          | 45.161                    | 5.012             | 0.797   | 0.077  | 6.866        | 11.203          | 25.000          | 44.81%                      |
|  | 2          | 62.191                    | 6.902             | 0.797   | 0.105  | 7.537        | 13.965          | 25.000          | 55.86%                      |
|  | 3          | 49.737                    | 5.520             | 0.797   | 0.084  | 7.231        | 11.658          | 25.000          | 46.63%                      |
|  | 4          | 56.593                    | 6.281             | 0.797   | 0.097  | 7.334        | 13.193          | 25.000          | 52.77%                      |
|  | 5          | 52.845                    | 5.865             | 0.798   | 0.090  | 7.994        | 11.199          | 25.000          | 44.80%                      |
| PG <sub>1000</sub> & TTA <sub>25</sub> | 8          | 39.056                    | 4.335             | 0.797   | 0.066  | 6.853        | 9.649           | 25.000          | 38.60%                      |
|  | 9          | 42.454                    | 4.712             | 0.800   | 0.071  | 8.204        | 8.651           | 25.000          | 34.60%                      |
|  | 10         | 44.555                    | 4.945             | 0.797   | 0.077  | 7.508        | 10.189          | 25.000          | 40.76%                      |
|  | 11         | 50.348                    | 5.588             | 0.798   | 0.086  | 7.843        | 10.975          | 25.000          | 43.90%                      |
|  | 12         | 56.618                    | 6.284             | 0.800   | 0.090  | 8.394        | 10.743          | 25.000          | 42.97%                      |

**Note:** All values of measurement (electronic scale or HPLC) were performed three times for each value represented in these data tables above.

Table H-6  
HPLC data for Tolyltriazole (250 mg/kg) Treatment of Uncontaminated Soil  
(After Respirometry Experiments)

| Treatment                               | Microcosm | Wt of 40 mL Vial (gm) | Wt Vial & Soil (gm) | Wt Vial & Soil & Methanol (gm) | Wt of Methanol in the Vial (gm) | Wt of Soil in the Vial (gm) |
|---|-----------|-----------------------|---------------------|--------------------------------|---------------------------------|-----------------------------|
| TTA <sub>250</sub>                      | 1         | 22.5350               | 34.3986             | 45.7780                        | 11.3794                         | 11.8636                     |
|   | 2         | 22.3719               | 35.5217             | 47.1501                        | 11.6284                         | 13.1498                     |
|   | 3         | 22.4041               | 36.2176             | 47.7770                        | 11.5594                         | 13.8135                     |
|   | 4         | 22.3553               | 33.1823             | 44.7836                        | 11.6013                         | 10.8270                     |
|   | 5         | 22.4222               | 36.2612             | 47.8345                        | 11.5733                         | 13.8390                     |
| PG <sub>1000</sub> & TTA <sub>250</sub> | 8         | 22.3768               | 35.8517             | 47.4285                        | 11.5768                         | 13.4749                     |
|   | 9         | 22.3548               | 35.3941             | 46.8474                        | 11.4533                         | 13.0393                     |
|   | 10        | 22.4244               | 35.8969             | 47.3368                        | 11.4399                         | 13.4726                     |
|   | 11        | 22.4282               | 33.4309             | 45.1015                        | 11.6706                         | 11.0027                     |
|   | 12        | 22.4331               | 33.6359             | 45.1128                        | 11.4770                         | 11.2028                     |

| Treatment                               | Microcosm | Wt of Alum Cont (gm) | Wt of Alum Cont & Wet Soil (gm) | Wt of Alum Cont & Dry Soil (gm) | Wt of Wet Soil (gm) | Wt of Dry Soil (gm) | Wt of H <sub>2</sub> O (gm) | % H <sub>2</sub> O in Spent Soil |
|---|-----------|----------------------|---------------------------------|---------------------------------|---------------------|---------------------|-----------------------------|----------------------------------|
| TTA <sub>250</sub>                      | 1         | 1.5644               | 12.0875                         | 10.9265                         | 10.5231             | 9.3621              | 1.1610                      | 11.03%                           |
|   | 2         | 1.5504               | 13.4043                         | 12.1118                         | 11.8539             | 10.5614             | 1.2925                      | 10.90%                           |
|   | 3         | 1.5521               | 12.9319                         | 11.5875                         | 11.3798             | 10.0354             | 1.3444                      | 11.81%                           |
|   | 4         | 1.5573               | 15.1907                         | 13.7298                         | 13.6335             | 12.1725             | 1.4610                      | 10.72%                           |
|   | 5         | 1.5588               | 13.5468                         | 12.1012                         | 11.9880             | 10.5424             | 1.4456                      | 12.06%                           |
| PG <sub>1000</sub> & TTA <sub>250</sub> | 8         | 1.5571               | 13.4280                         | 12.1117                         | 11.8709             | 10.5546             | 1.3163                      | 11.09%                           |
|   | 9         | 1.5547               | 12.7645                         | 11.5791                         | 11.2099             | 10.0244             | 1.1855                      | 10.58%                           |
|   | 10        | 1.5566               | 13.4392                         | 11.9261                         | 11.8826             | 10.3695             | 1.5131                      | 12.73%                           |
|   | 11        | 1.5564               | 15.3807                         | 13.8154                         | 13.8243             | 12.2590             | 1.5653                      | 11.32%                           |
|   | 12        | 1.5524               | 13.9648                         | 12.5435                         | 12.4124             | 10.9910             | 1.4214                      | 11.45%                           |

| Treatment                               | Microcosms | Average HPLC Area (mAu*s) | Conc.             | Density of Methanol/H <sub>2</sub> O mix in Vial    | Mass of Toly in Vial                                       | Soil in Vial | End Conc        | Initial Conc    | % recovered                 |
|---|------------|---------------------------|-------------------|---|--|--------------|-----------------|-----------------|-----------------------------|
|   |            | y = 9.01x                 | x = y/9.01 (mg/L) | $\frac{(wtH_2O+wtMeth)}{(volH_2O+volMeth)}$ (mg/mL) | $\frac{((conc/density)*wtH_2O + Meth))/1000mL}{(mg toly)}$ | (mg)         | mg toly kg soil | mg toly kg soil | (End Conc/Initial Conc)*100 |
| TTA <sub>250</sub>                      | 1          | 1308.394                  | 145.216           | 0.807   | 2.284  | 11.864       | 192.559         | 250.000         | 77.02%                      |
|   | 2          | 1581.670                  | 175.546           | 0.808   | 2.839  | 13.150       | 215.890         | 250.000         | 86.36%                      |
|   | 3          | 1533.080                  | 170.153           | 0.810   | 2.771  | 13.813       | 200.568         | 250.000         | 80.23%                      |
|   | 4          | 1218.012                  | 135.184           | 0.804   | 2.145  | 10.827       | 198.075         | 250.000         | 79.23%                      |
|   | 5          | 1616.160                  | 179.374           | 0.811   | 2.930  | 13.839       | 211.753         | 250.000         | 84.70%                      |
| PG <sub>1000</sub> & TTA <sub>250</sub> | 8          | 1399.876                  | 155.369           | 0.809   | 2.512  | 13.475       | 186.409         | 250.000         | 74.56%                      |
|   | 9          | 1432.343                  | 158.973           | 0.807   | 2.527  | 13.039       | 193.790         | 250.000         | 77.52%                      |
|   | 10         | 1284.401                  | 142.553           | 0.811   | 2.311  | 13.473       | 171.568         | 250.000         | 68.63%                      |
|   | 11         | 1123.405                  | 124.684           | 0.805   | 2.000  | 11.003       | 181.739         | 250.000         | 72.70%                      |
|   | 12         | 1175.323                  | 130.447           | 0.806   | 2.065  | 11.203       | 184.315         | 250.000         | 73.73%                      |

**Note:** All values of measurement (electronic scale or HPLC) were performed three times for each value represented in these data tables above.

A summarization of Tables H-4 through H-6 is provided in Table H-7 below.

Table H-7  
Percentages of Tolyltriazole Residual Recovered

| Treatment                               | Percent of tolyltriazole residual measured through HPLC analysis |         |           |   |         |           |
|---|--|---------|-----------|---|---------|-----------|
|   | Before Respirometry Test (3 samples used)                        |         |           | After Respirometry Test (5 microcosms used) |         |           |
|   | Avg  | Std Dev | Reference | Avg   | Std Dev | Reference |
| TTA <sub>25</sub>                       | 99.79%   | 1.35%   | Table H-4 | 48.97%                                      | 5.05%   | Table H-5 |
| TTA <sub>250</sub>                      | 90.56%   | 0.33%   | Table H-4 | 81.51%                                      | 3.89%   | Table H-6 |
| TTA <sub>500</sub>                      | 95.15%   | 0.08%   | Table H-4 | No test performed                           | -----   | -----     |
| PG <sub>1000</sub> & TTA <sub>25</sub>  | 97.21%   | 1.17%   | Table H-4 | 40.17%                                      | 3.73%   | Table H-5 |
| PG <sub>1000</sub> & TTA <sub>250</sub> | 95.59%   | 0.17%   | Table H-4 | 73.43%                                      | 3.23%   | Table H-6 |
| PG <sub>1000</sub> & TTA <sub>500</sub> | 95.93%   | 0.12%   | Table H-4 | No test performed                           | -----   | -----     |

#### Statistical Analysis of Percent Tolyltriazole Recovered

The recovered tolyltriazole after respirometry tests appears to have a lower by a difference of  $\sim 8.5\% \Delta \pm \text{Std Dev}$  when in the presence of propylene glycol (Table H-8).

Table H-8  
Difference in Tolyltriazole Percentage Recovered due to Propylene Glycol Presence

| Treatment                               | Percent of tolyltriazole residual measured through HPLC analysis After Respirometry Test (5 microcosms used) |         |  |
|---|--|---------|--|
|   | Avg  | Std Dev |  |
| TTA <sub>25</sub>                       | 48.97%   | 5.05%   | <div style="border: 1px dashed black; padding: 5px; display: inline-block;"> <math>8.8\% \Delta \pm \text{Std}</math> </div> |
| TTA <sub>250</sub>                      | 81.51%   | 3.89%   |  |
| TTA <sub>500</sub>                      | No test performed  | -----   |  |
| PG <sub>1000</sub> & TTA <sub>25</sub>  | 40.17%   | 3.73%   | <div style="border: 1px solid black; padding: 5px; display: inline-block;"> <math>8.1\% \Delta \pm \text{Std}</math> </div>  |
| PG <sub>1000</sub> & TTA <sub>250</sub> | 73.43%   | 3.23%   |  |
| PG <sub>1000</sub> & TTA <sub>500</sub> | No test performed  | -----   |  |

The indication was that the tolyltriazole mass (25 mg/kg or 250 mg/kg) degraded at a consistent amount ( $((8.8\% + 8.1\%)/2 = \sim 8.5\%)$ ) when present with propylene glycol (1,000 mg/kg) in the soil. A two-sample t-test was used to identify if theses additional degradation percentages (8.1% and 8.8%) were similar, or if the standard deviations would dismiss the possibility.

### Two sample t-test set-up

A two sample t-test, with a significance level of ( $\alpha = 0.05$ ) was used. The null hypothesis stated below [Devore, 357-360].

$H_0$ : The null hypothesis was that the additional degradation percentages (8.1% and 8.8%) were similar in value for the two different treatments of TTA

$H_a$ : The additional degradation percentages were not similar in value (due to Std Dev)

$$H_0: \mu_D = \Delta_0$$

$$\mu_D = \mu_1 - \mu_2$$

$\Delta_0$  = The differences of the pairs  $\approx$  zero

$$H_a: \mu_D \neq \Delta_0$$

### Data:

|   |   |        |        |        |        |        | Average | Std Dev |
|---|---|--------|--------|--------|--------|--------|---------|---------|
| 1 | TTA <sub>25</sub>                       | 0.4480 | 0.4481 | 0.4663 | 0.5277 | 0.5586 | 0.0881  | 0.02653 |
|   | PG <sub>1000</sub> & TTA <sub>25</sub>  | 0.3460 | 0.3860 | 0.4076 | 0.4297 | 0.4390 |         |         |
|   | Difference =                            | 0.1019 | 0.0621 | 0.0588 | 0.0980 | 0.1196 |         |         |
| 2 | TTA <sub>250</sub>                      | 0.7702 | 0.7923 | 0.8023 | 0.8470 | 0.8636 | 0.0808  | 0.01565 |
|   | PG <sub>1000</sub> & TTA <sub>250</sub> | 0.6863 | 0.7270 | 0.7373 | 0.7456 | 0.7752 |         |         |
|   | Difference =                            | 0.0840 | 0.0653 | 0.0650 | 0.1014 | 0.0884 |         |         |

### Test statistic value:

$$t = \frac{\bar{x}_{\text{bar}} - \bar{y}_{\text{bar}} - \Delta_0}{S_p(1/n_1 + 1/n_2)^{1/2}}$$

$$S_p^2 = \frac{(n_1 - 1) * S_1^2 + (n_2 - 1) * S_2^2}{(n_1 + n_2) - 2} = .00004743817$$

$n_1$  = number of differences TTA<sub>25</sub> = 5

$n_2$  = number of differences TTA<sub>250</sub> = 5

$$t = \frac{(.0881 - .0808) - 0}{.002178(1/5 + 1/5)^{1/2}}$$

$$t = 0.587$$

$$t_{\text{crit value}} = t_{\alpha/2, (n_1 + n_2) - 2} = 2.306 \text{ [Devore, 707]}$$

### Rejection region for level of test

$t \geq t_{\text{crit}} = \text{Reject the null}$

$t \leq -t_{\text{crit}} = \text{Reject the null}$

$$.0587 \leq 2.306$$

$t \leq t_{\text{crit}}$ , thus we do not reject the null, and say that the additional degradation percents for the two different treatments were similar

## Appendix I: Microbial Colony Population Count Results

Table I-1

Averaged Microbial Colony Population Counts (48 hr point) from Interaction with Respirometry Soil (Run-2), Chemical Concentrations of Propylene Glycol (1,000 mg/kg) and Tolyltriazole (250 mg/kg)

| Dilution (mL) | Microbial Colony Populations Counted |                    |                    |   |
|---------------|--------------------------------------|--------------------|--------------------|---|
|               | Blank                                | TTA <sub>250</sub> | PG <sub>1000</sub> | PG <sub>1000</sub> & TTA <sub>250</sub> |
| 0.01          | >300                                 | >300               | >300               | >300                                    |
| 0.001         | 52                                   | 125                | 161                | 193                                     |
| 0.0001        | 15                                   | 32                 | 14                 | 111                                     |
| 0.00001       | 1                                    | 1                  | 3                  | 6                                       |

Table I-2

Averaged Microbial Colony Population Counts (48 hr point) from Interaction with Respirometry Soil (Run-3), Chemical Concentrations of Propylene Glycol (1,000 mg/kg) and Tolyltriazole (500 mg/kg)

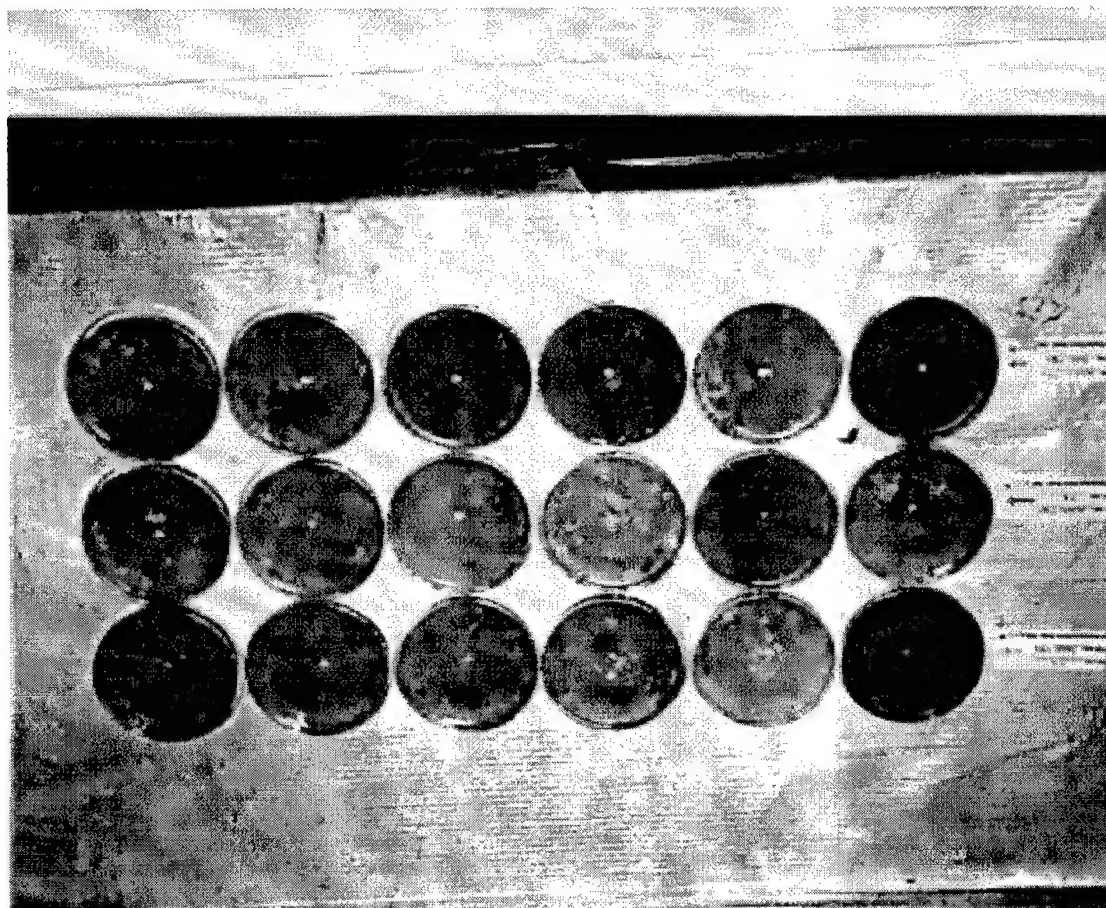
| Dilution (mL) | Microbial Colony Populations Counted |                    |                    |   |
|---------------|--------------------------------------|--------------------|--------------------|---|
|               | Blank                                | TTA <sub>500</sub> | PG <sub>1000</sub> | PG <sub>1000</sub> & TTA <sub>500</sub> |
| 0.01          | >300                                 | >300               | >300               | >300                                    |
| 0.001         | >300                                 | >300               | >300               | >300                                    |
| 0.0001        | 110                                  | 117                | 201                | 231                                     |
| 0.00001       | 14                                   | 11                 | 27                 | 16                                      |

**Note:** Each MCPC listed (Tables I-1 and Table I-2) used three replicates, counted three times and averaged.



## Appendix J: Agar Well Diffusion Test Results

Figure J-1  
AWDT Visual Results (November 01, 1998)



**Note:** The white spots/areas represent uncolonized nutrient agar. There were no signs of inhibition on microbial colony growth around the well area.

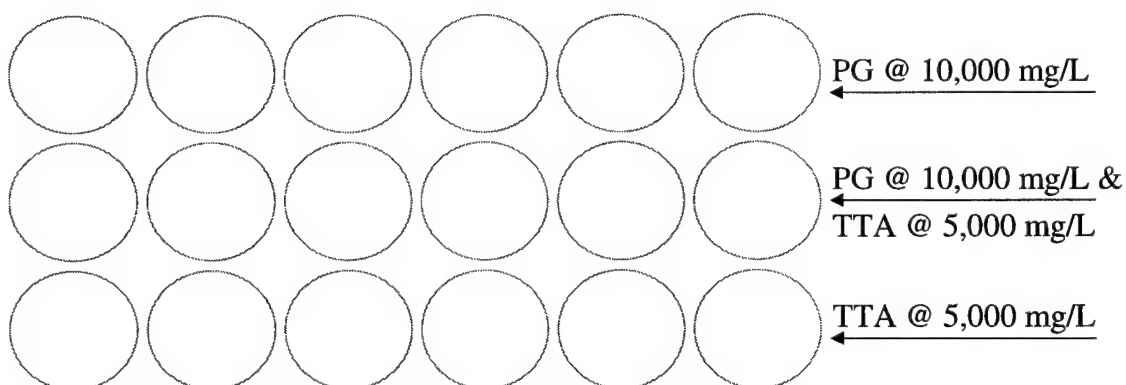
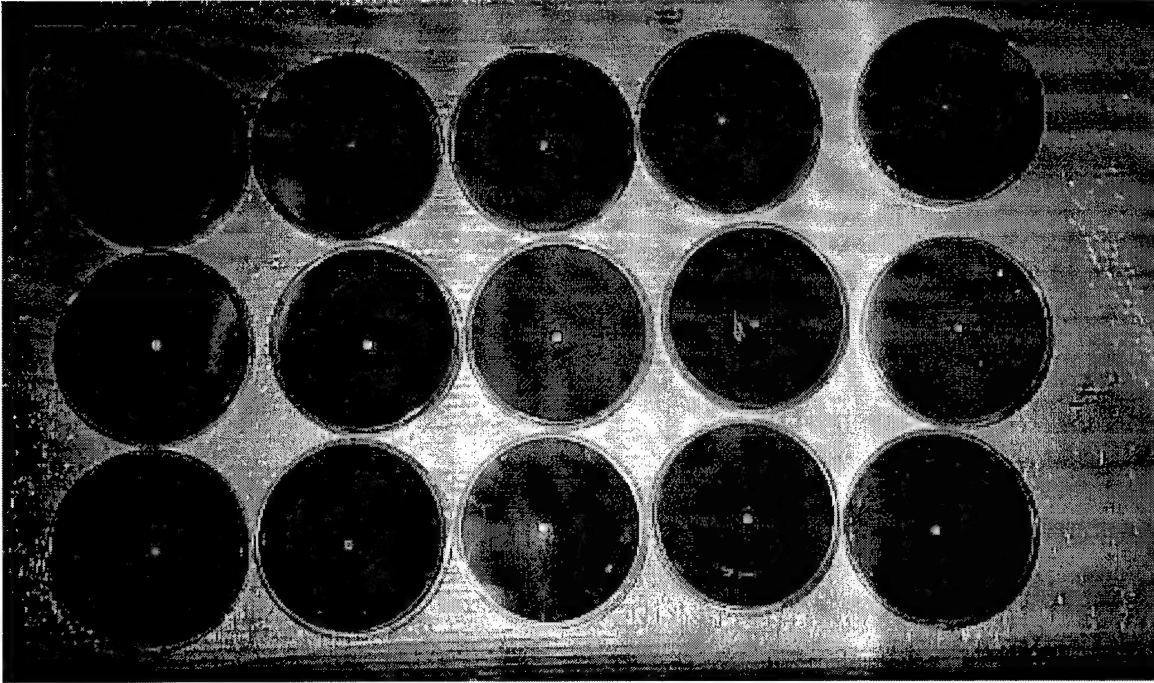
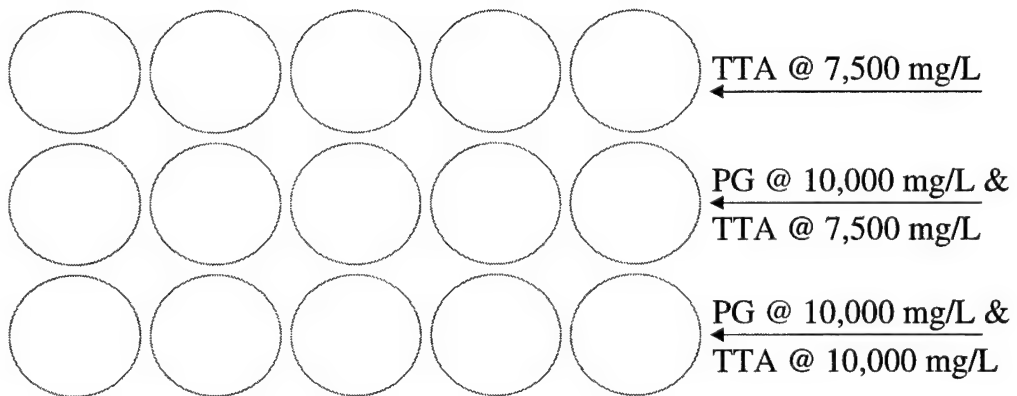


Figure J-2  
AWDT Visual Results (November 29, 1998)



**Note:** The white spots/areas represent uncolonized nutrient agar. There were no signs of inhibition on microbial colony growth around the well area.



## Appendix K: Theoretical Oxygen Demand Calculations

Theoretical oxygen demand (ThOD) calculations were generated from the O<sub>2</sub> consumption totals at the 336 hr and 468 hr points. Table K-1 summarizes the values.

Table K-1  
"Actual" O<sub>2</sub> Consumption Totals for ThOD Calculations

| Treatment                                | Time point<br>O <sub>2</sub> totals | Avg O <sub>2</sub><br>total (uL) | Std Dev O <sub>2</sub><br>total (uL) | Blank O <sub>2</sub><br>total (uL) | V <sub>act</sub><br>(Actual - Blank) |              |
|--|-------------------------------------|----------------------------------|--------------------------------------|------------------------------------|--------------------------------------|--------------|
|  |                                     |                                  |                                      |                                    | Avg (uL)                             | Std Dev (uL) |
| PG <sub>1000</sub>                       | 336 hr                              | 37678                            | 786                                  | 8808                               | 29054                                | 786          |
| PG <sub>1000</sub> & TTA <sub>25</sub>   | 468 hr                              | 44157                            | 1428                                 | 10523                              | 33633                                | 1428         |
| PG <sub>1000</sub> & TTA <sub>25</sub>   | 336 hr                              | 41397                            | 993                                  | 8992                               | 32405                                | 993          |
| PG <sub>1000</sub> & TTA <sub>250</sub>  | 336 hr                              | 49516                            | 1898                                 | 8808                               | 41862                                | 1898         |
| PG <sub>1000</sub> & TTA <sub>500</sub>  | 336 hr                              | 52776                            | 1716                                 | 8624                               | 44152                                | 1716         |
| PG <sub>1000</sub> & TTA <sub>750</sub>  | 468 hr                              | 55491                            | 2190                                 | 10523                              | 44967                                | 2190         |
| PG <sub>1000</sub> & TTA <sub>1000</sub> | 468 hr                              | 32933                            | 2463                                 | 10523                              | 22410                                | 2463         |

**Note:** PG<sub>1000</sub> & TTA<sub>25</sub> was measured at both the 336 hr and 468 hr point to show percent biodegradation was similar (see Table K-3), using the Actual - Blank (O<sub>2</sub> consumption totals), thus allowing either time point to be used.

The ThOD equation for individual ADF chemical components; propylene glycol and tolyltriazole are listed in Tables 2-1 and Table 2-2, respectively. The calculation for converting milligrams (mg) to microliters (uL) of O<sub>2</sub> used the Ideal Gas Law. Atmospheric pressure was assumed at P = 1.00, and temperature (T) = 25°C from the respirometry runs.

Ideal Gas Law:

$$n = PV/RT$$

|                          |                         |                                |
|--------------------------|-------------------------|--------------------------------|
| V = Liters (Unknown)     | T = (273 + 25°C) Kelvin | n - moles O <sub>2</sub>       |
| L = 1x10 <sup>6</sup> uL | R = .082058 L*atm/K*mol | MW O <sub>2</sub> = 32 gm/mole |

Table K-2

"Total" ThOD for Available ADF Chemical Biodegradation on Uncontaminated Soil

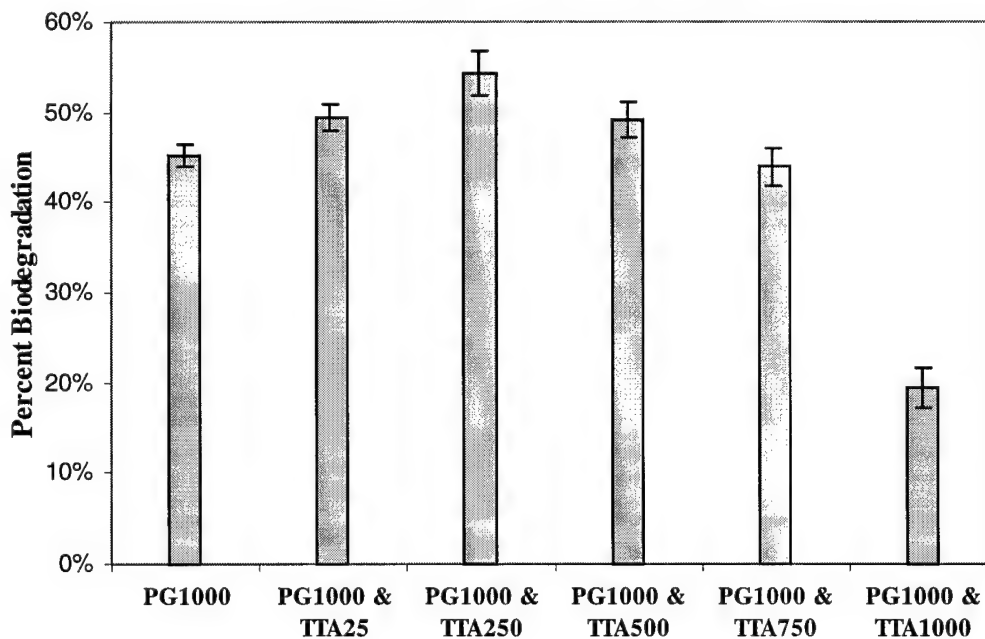
| Treatment                                | Mass of Chemical Available |          | ThOD for PG                      |       | ThOD for TTA                      |       | Total ThOD<br>(uL) |
|--|----------------------------|----------|----------------------------------|-------|-----------------------------------|-------|--------------------|
|  | PG (mg)                    | TTA (mg) | 1.682 mg O <sub>2</sub><br>mg PG | (uL)  | 1.564 mg O <sub>2</sub><br>mg TTA | (uL)  |                    |
| PG <sub>1000</sub>                       | 50                         | 0        | 84.10                            | 64266 | 0                                 | 0     | 64266              |
| PG <sub>1000</sub> & TTA <sub>25</sub>   | 50                         | 1.25     | 84.10                            | 64266 | 1.66                              | 1270  | 65537              |
| PG <sub>1000</sub> & TTA <sub>250</sub>  | 50                         | 12.5     | 84.10                            | 64266 | 16.63                             | 12704 | 76971              |
| PG <sub>1000</sub> & TTA <sub>500</sub>  | 50                         | 25       | 84.10                            | 64266 | 33.25                             | 25408 | 89675              |
| PG <sub>1000</sub> & TTA <sub>750</sub>  | 50                         | 37.5     | 84.10                            | 64266 | 49.88                             | 38113 | 102379             |
| PG <sub>1000</sub> & TTA <sub>1000</sub> | 50                         | 50       | 84.10                            | 64266 | 66.50                             | 50817 | 115083             |

The percentage of biodegradation was generated from  $V_{act}/\text{Total ThOD}$ . Table K-3 and Figure K-1 summarize the results.

Table K-3  
Percent Biodegradation from ThOD of Available ADF Chemical  
Components on Uncontaminated Soil

| Treatment                                | Total ThOD | $V_{act}$       |                 | % Biodegradation |         | Time point<br>O <sub>2</sub> totals |         |
|--|------------|-----------------|-----------------|------------------|---------|-------------------------------------|---------|
|  | (uL)       | Average<br>(uL) | Std Dev<br>(uL) | Average          | Std Dev |                                     |         |
| PG <sub>1000</sub>                       | 64266      | 29054           | 786             | 45%              | 1.2%    | 336 hr                              |         |
| PG <sub>1000</sub> & TTA <sub>25</sub>   | 65537      | 33633           | 1428            | 51%              | 2.2%    | 468 hr                              |         |
| PG <sub>1000</sub> & TTA <sub>25</sub>   | 65537      | 32405           | 993             | 49%              | 1.5%    | 336 hr                              | similar |
| PG <sub>1000</sub> & TTA <sub>250</sub>  | 76971      | 41862           | 1898            | 54%              | 2.5%    | 336 hr                              |         |
| PG <sub>1000</sub> & TTA <sub>500</sub>  | 89675      | 44152           | 1716            | 49%              | 1.9%    | 336 hr                              |         |
| PG <sub>1000</sub> & TTA <sub>750</sub>  | 102379     | 44967           | 2190            | 44%              | 2.1%    | 468 hr                              |         |
| PG <sub>1000</sub> & TTA <sub>1000</sub> | 115083     | 22410           | 2463            | 19%              | 2.1%    | 468 hr                              |         |

Figure K-1  
Percent Biodegradation from ThOD of Available ADF Chemical  
Components on Uncontaminated Soil



Biodegradation rates in terms of mass of soil were calculated for the propylene glycol application on soil. Shown below is a sample calculation, which used Run-1, bottle/microcosm 16.

|   |   |
|---|---|
| $\text{inter} := 6 \cdot \text{hr}$   | Time per sampling interval, one sample per 6 hours  |
| $\text{number}_{\text{interval}} := 56$   | Number of intervals under investigation/shown below   |
| $\text{hours}_{\text{exp}} := \text{inter} \cdot 56$                              | Number of hours in the experiment run = 336 hours   |
| $\text{hours}_{\text{exp}} = 336 \cdot \text{hr}$                                 |   |
| $v := 38996$  | Microliters of oxygen consumed in treatment (336 hrs)   |
| $v_{\text{soil\_blank}} := 8808$  | Microliters of oxygen (uL) from blank soil <u>averaged</u> (336 hrs)  |
| $v_{\text{act}} := v - v_{\text{soil\_blank}}$                                    | Adjusting for background oxygen readings from blank soil (de-ionized H <sub>2</sub> O on soil)  |
| $V := \left( \frac{v_{\text{act}}}{1000000} \right) \cdot \text{L}$               | Conversion of Microliters to Liters   |
| $P := 1 \cdot \text{atm}$   | Standard atmospheric pressure (atm)   |
| $t := 25$   | Temperature of respirometry tests (°C)  |
| $T := (273 + t) \cdot \text{K}$   | Conversion to Kelvin (°K)   |
| $R := 0.082058 \cdot \frac{\text{L} \cdot \text{atm}}{\text{K} \cdot \text{mol}}$ | Gas Constant (L-atm/deg K-mol)  |
| $n := \frac{P \cdot V}{R \cdot T}$  | Ideal Gas Law   |
| $n = 0.0012 \cdot \text{mol}$   | The number of moles of oxygen consumed  |
| $\text{soil} := .050 \cdot \text{kg}$   | Weight of ~60% FC soil (kg) in each microcosm   |
| $\text{resp\_rate} := \frac{\frac{V}{\text{hours}_{\text{exp}}}}{\text{soil}}$    | $\text{resp\_rate} = 0.02995 \cdot \frac{\text{mL}}{\text{min} \cdot \text{kg}}$ <b>Respiration Rate (mL/min/kg)</b><br><b>~60% FC soil</b> |
| $\text{ratio} := 4$   | Number of moles O <sub>2</sub> required to mineralize 1 mole C <sub>3</sub> H <sub>8</sub> O <sub>2</sub>                                   |
| $\text{MW} := 76.094 \cdot \frac{\text{gm}}{\text{mole}}$                         | Molecular weight of C <sub>3</sub> H <sub>8</sub> O <sub>2</sub> (gm/mole)  |
| $\text{mass}_{\text{PG}} := \frac{n}{\text{ratio}} \cdot \text{MW}$               | $\text{mass}_{\text{PG}} = 23.48 \cdot \text{mg}$ <b>Mass of PG, Consumed (mg)</b>  |

$$\text{mass PG}_{\text{orig}} := 50.0 \text{ mg}$$

Original mass of PG in solution added to soil

[5 mL of 10,000 PG mg/L = 50 mg PG added to 50 gm soil]

$$\text{percent lost} := \left( \frac{\text{mass PG}}{\text{mass PG}_{\text{orig}}} \right) \cdot 100$$

$$\text{percent lost} = 46.97$$

**% PG Lost to Biodegradation**

$$\text{spgr}_{\text{hc}} := 1.0 \frac{\text{mL}}{\text{mg}}$$

Specific gravity solution is considered to be 1.00 ml/mg  
since PG solution is mainly composed of de-ionized water

$$\text{degrade rate} := \frac{\left( \frac{\text{mass PG} \cdot \text{spgr}_{\text{hc}}}{\text{hours exp}} \right)}{\text{soil}}$$

$$\text{degrade rate} = 33.55 \text{ kg}^{-1} \frac{\text{mL}}{\text{day}}$$

**PG Biodegradation Rate, ml/day  
kg soil**

# **Appendix L: Statistical Procedures for Determining the Difference of Initial Biodegradation Rates of Uncontaminated Soil (Phase-one) compared to Acclimated Soil (Phase-two)**

## **Overview of Statistical Test:**

The statistical testing used a two-sided t-test to identify the biodegradation rates difference due to ADF components application on acclimatized microorganism/soil vs uncontaminated microorganism/soil. The statistical test used significance level of  $\alpha = 0.05$ .

$H_0$ : There was no difference between initial biodegradation rates from PG<sub>1000</sub> treatment of uncontaminated compared to acclimated soil

$H_a$ : There was a difference between initial biodegradation rates from PG<sub>1000</sub> treatment of uncontaminated compared to acclimated soil

## **Data and Calculations Performed prior to Statistical Test:**

The biodegradation rates were calculated from equations used in Appendix K for the total time of 24 hrs. Run-1, Run-2, and Run-3 data was used to represent the uncontaminated soil inoculated with PG<sub>1000</sub> used (15 replicates). Run-6 data was used to represent the PG<sub>1000</sub> on acclimated PG<sub>1000</sub> soil (5 replicates).

**Note:** The two soil types used blank tests (de-ionized water applied to the soil type) to measure any unusual respiration activity. The difference of the propylene glycol treatment minus the average blank (de-ionized H<sub>2</sub>O) treatment was the O<sub>2</sub> total used for initial biodegradation rate. The calculations in Appendix K were used to generate the biodegradation rates per mass of soil.

## **Data:**

Table L-1  
Cumulative O<sub>2</sub> Consumption (336 hr point) Data for PG<sub>1000</sub> Treatment  
on Acclimated and Uncontaminated Soil

| O <sub>2</sub> Totals (24 hr point) |  |  |   |  |
|-------------------------------------|--|--|---|--|
| (uL)                                |  |  |   |  |
| Run                                 | PG <sub>1000</sub><br>on<br>Uncontaminated | Run-1 & 2 & 3<br>De-ionized H <sub>2</sub> O<br>on<br>Uncontaminated | Run-6<br>PG <sub>1000</sub><br>on<br>Acclimated | Run-6<br>De-ionized H <sub>2</sub> O<br>on<br>Acclimated |
| 1                                   | 7525                                       | 1401   | 9741  | 1045   |
| 1                                   | 8190                                       | 1401   | 11199   | 1045   |
| 1                                   | 7183                                       | 1401   | 11532   | 1045   |
| 1                                   | 7468                                       | 1401   | 11452   | 1045   |
| 1                                   | 8111                                       | 1401   | 10903   | 1045   |
| 2                                   | 7583                                       | 1401   |   |  |
| 2                                   | 8483                                       | 1401   |   |  |
| 2                                   | 8220                                       | 1401   |   |  |
| 2                                   | 8518                                       | 1401   |   |  |
| 2                                   | 8316                                       | 1401   |   |  |
| 3                                   | 9438                                       | 1401   |   |  |
| 3                                   | 8939                                       | 1401   |   |  |
| 3                                   | 9214                                       | 1401   |   |  |
| 3                                   | 8870                                       | 1401   |   |  |
| 3                                   | 8508                                       | 1401   |   |  |

->  
Calc

| Biodegradation Rates |  |   |
|----------------------|--|---|
| (mL/day/kg soil)     |  |   |
| Run                  | PG <sub>1000</sub><br>on<br>Uncontaminated | Run-6<br>PG <sub>1000</sub><br>on<br>Acclimated |
| 1                    | 95.28                                      | 129.76  |
| 1                    | 105.63                                     | 152.45  |
| 1                    | 89.96                                      | 157.63  |
| 1                    | 94.39                                      | 156.38  |
| 1                    | 104.40                                     | 147.84  |
| 2                    | 96.18                                      |   |
| 2                    | 110.19                                     |   |
| 2                    | 106.09                                     |   |
| 2                    | 110.73                                     |   |
| 2                    | 107.58                                     |   |
| 3                    | 125.05                                     |   |
| 3                    | 117.28                                     |   |
| 3                    | 121.56                                     |   |
| 3                    | 116.21                                     |   |
| 3                    | 110.58                                     |   |

### Wilk-Shapiro/Rankit Plot

STATISTIX<sup>®</sup> 4.0 software was used to test the distributions of each population. The test was performed to demonstrate the approximate normality of the data. Figures L-1 and Figure L-2 plots the normality for unacclimated soil and acclimated soil, respectively.

Figure L-1  
Wilk-Shapiro/Rankit Plot of Initial Biodegradation Rates (24 hr point)  
from PG<sub>1000</sub> Interaction with Uncontaminated Soil

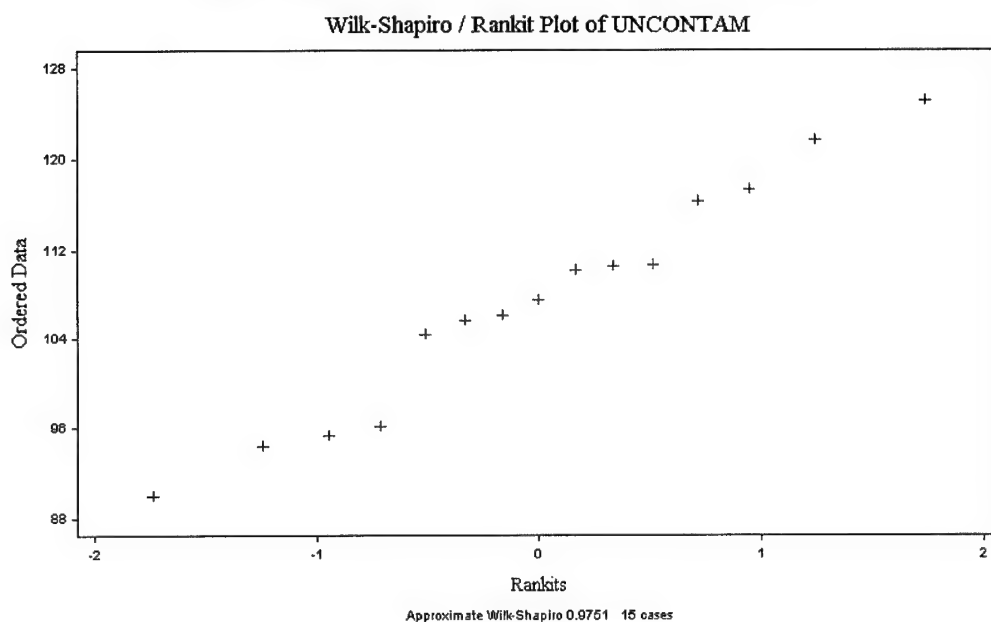
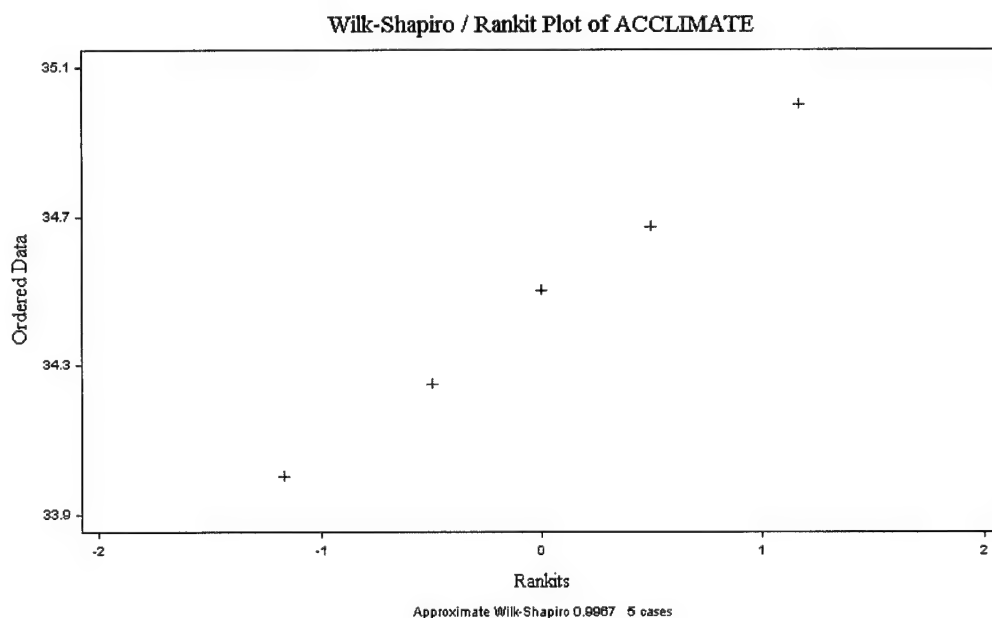


Figure L-2  
Wilk-Shapiro/Rankit Plot of Initial Biodegradation Rates (24 hr point)  
from PG<sub>1000</sub> Interaction with Acclimated Soil (PG<sub>1000</sub>)





**Statistical Test:**

The distribution of the initial biodegradation rates was approximately normal in both figures. Thus, the t-test can be performed. The averaged initial biodegradation rates for the PG<sub>1000</sub> on uncontaminated soil was set as the standard mean. The mean of the initial biodegradation rates for PG<sub>1000</sub> on acclimated soil (PG<sub>1000</sub>) was compared to the standard mean.

**Tests Statistic Value (t\*):**

$$t = \frac{\bar{x} - \mu_0}{(s/n^{0.5})} \quad (\text{Devore, 291})$$

$\bar{x}$  = Mean of acclimated soil results (bio-rate)

$\mu_0$  = Mean of uncontaminated soil results (bio-rate)

s = Standard deviation of acclimated soil results

n = Number of replicates

**T- Critical Value (t<sub>crit</sub>):**

T-critical (t<sub>crit</sub>) was determined for a two-tailed test since the effects on biodegradation rates may be enhanced or inhibited as the alternate hypothesis. The ultimate decision of biodegradation, no effect, or inhibition was made by comparing the t-statistic to the t-critical.

$$t_{crit} = t_{\alpha/2, n-1} = \pm 2.447 \quad (\text{Value from Table A.5, Devore, 707})$$

$$\alpha = 0.05$$

$$n = 5 \text{ (replicates)}$$

**Rejection Region:**

$$-t_{crit} \leq t^* \leq t_{crit}$$

$$-2.447 \leq t^* \leq 2.447$$

If the t\* value falls between the t<sub>crit</sub> values, do not reject H<sub>0</sub>  
(Devore, 318)

**Summarization of Results:**

Table L-2

Statistical Test of Acclimated versus Uncontaminated Soil Initial Biodegradation Rates

| Averaged<br>(Uncontaminated) | Average<br>(Acclimated) | Std Dev<br>(Acclimated) | Replicates<br>(Acclimated) | t-value  | t-critical<br>value |                       |
|------------------------------|-------------------------|-------------------------|----------------------------|----------|---------------------|-----------------------|
| $\mu_0$                      | $\bar{x}$               | s                       | n                          | t*       | t <sub>crit</sub>   | Reject H <sub>0</sub> |
| 107.41                       | 148.81                  | 11.3149565              | 5                          | 27.52337 | 2.776               | Yes                   |

The null hypothesis was rejected. The conclusion was a significant increase in the initial biodegradation rates when PG<sub>1000</sub> was applied on acclimated soil (with PG<sub>1000</sub>) compared to the biodegradation rates from PG<sub>1000</sub> application on uncontaminated soil.

## Appendix M: Statistical Procedures for Testing the Quality/Repeatability of Data from Laboratory/Respirometry Runs

### **Overview of Test**

The statistical analysis used a one-way ANOVA for testing the quality of laboratory procedure and the respirometry measurements through identical treatments used in the respirometry runs. The means of O<sub>2</sub> consumption totals, at the specific time point of 288 hrs, was used to perform the ANOVA comparisons.

There were two types of soil treatments evaluated (separately) with the statistical analysis.

1. Blank/De-ionized water on soil was performed in Run-1, Run-2, and Run-3 was used to measure the respirometers measurement quality.
  - A total of three (or more) microcosms/samples were available in each run
2. PG<sub>1000</sub> application on soil was performed in Run-1 through Run-5 was used to measure the laboratory procedures/technique quality.
  - A total of three (or more) microcosm/samples were available in each run

The statistical test used a significance level of  $\alpha = 0.05$

H<sub>0</sub>: There was no difference between respirometry data sets using the same respirometer/laboratory procedures

H<sub>a</sub>: There was a difference between (one or all) respirometry data sets using the same respirometer/laboratory procedures

$$H_0 = \mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5$$

$$H_a = \mu_1 \neq \mu_{i...5}$$

### **Data: Means of Cumulative O<sub>2</sub> (μL) from Each Experimental Run**

Table M-1  
Cumulative O<sub>2</sub> Consumption (288 hr point) Data for De-ionized  
H<sub>2</sub>O and PG<sub>1000</sub> Treatments on Uncontaminated Soil

|       | De-ionized H <sub>2</sub> O on Uncontaminated Soil |      |      |  |  | Average | Std Dev |
|-------|--|------|------|--|--|---------|---------|
| Run-1 | 8259   | 8587 | 7947 |  |  | 8264    | 320     |
| Run-2 | 7741   | 8526 | 7877 |  |  | 8048    | 420     |
| Run-3 | 7681   | 8394 | 7569 |  |  | 7881    | 448     |

|       | PG <sub>1000</sub> on Uncontaminated Soil |       |       |       |       | Average | Std Dev |
|-------|---|-------|-------|-------|-------|---------|---------|
| Run-1 | 37907                                     | 37092 | 36117 | 37865 | 38773 | 37551   | 998     |
| Run-2 | 43787                                     | 43530 | 46398 | 46142 | 44508 | 44873   | 1328    |
| Run-3 | 36319                                     | 35220 | 35318 | 36193 | 35963 | 35803   | 505     |
| Run-4 | 36455                                     | 37469 | 36587 |       |       | 36837   | 551     |
| Run-5 | 35282                                     | 38451 | 38062 |       |       | 37265   | 1729    |

**Test Statistic:**

The test statistic is  $F_{\alpha, v1, v2} = F_{crit}$  (Devore 709)

|                                 | De-ionized H <sub>2</sub> O on soil | PG <sub>1000</sub> on soil |
|---------------------------------|-------------------------------------|----------------------------|
| Treatments number (J)           | 3                                   | 5                          |
| Sample size (I)                 | 3                                   | 5                          |
| Formula degree freedom          |                                     |                            |
| $v1 = I - 1$                    | 2                                   | 4                          |
| $v2 = I(J - 1)$                 | 6                                   | 20                         |
| Info/formula above = $F_{crit}$ | 5.14                                | 2.87                       |

**Decision Rule:**

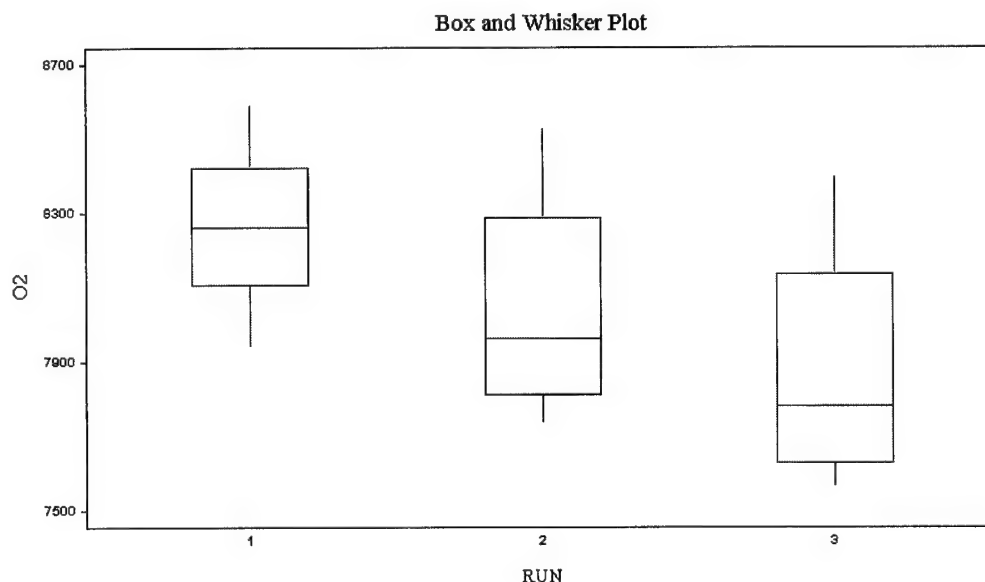
If  $f^* \geq F_{\alpha, v1, v2}$  then reject the null hypothesis, else do not reject, or  
 If  $P\text{-value} \leq \alpha$  then reject the null hypothesis, else do not reject  
 Formula  $f^* = \text{MSEr}/\text{MSE}$

The computation of  $f^*$  and relevant statistical testing data were performed with the STATISTIX<sup>®</sup> 4.1 software. The results are shown below for the two different types of soil treatments (de-ionized H<sub>2</sub>O or PG<sub>1000</sub>).

**STATISTIX<sup>®</sup> Results for De-ionized H<sub>2</sub>O on Uncontaminated soil**

Outliers were checked on the data sets using a Box and Whisker plot as shown in Figure M-1.

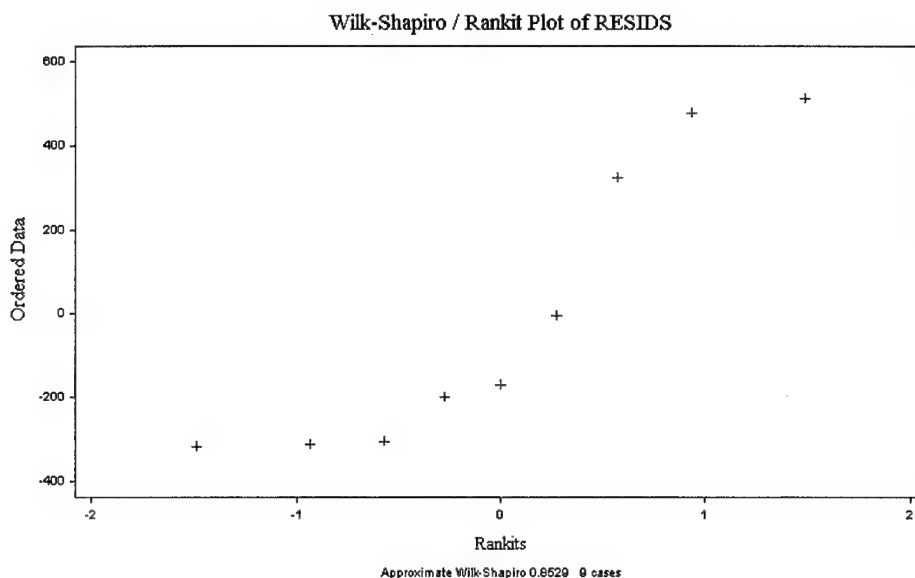
Figure M-1  
 Box-Whiskers Plot of O<sub>2</sub> totals for De-ionized H<sub>2</sub>O on Uncontaminated Soil  
 (288 hr point)



**Note:** No outliers were apparent in any of the respirometry runs (data sets).

The one-way ANOVA produced the residuals for the three different respirometry runs. The residuals were plotted using a Wilk-Shapiro/Rankit plot as shown in Figure M-2.

Figure M-2  
Wilk-Shapiro/Rankit Plot of Residuals for De-ionized H<sub>2</sub>O on Uncontaminated Soil  
(288 hr point)



The residuals show aptness ( $R = 0.853$ ), thus statistical testing was continued with the one-way ANOVA results, as shown in Table M-2.

Table M-2  
One-way ANOVA results for De-ionized H<sub>2</sub>O on Uncontaminated Soil  
(288 hr point)

| ONE-WAY AOV FOR O2 BY RUN             |    |         |        |             |               |
|---------------------------------------|----|---------|--------|-------------|---------------|
| SOURCE                                | DF | SS      | MS     | F           | P             |
| BETWEEN                               | 2  | 221267  | 110633 | <b>0.69</b> | <b>0.5359</b> |
| WITHIN                                | 6  | 957329  | 159555 |             |               |
| TOTAL                                 | 8  | 1178596 |        |             |               |
|                                       |    |         |        |             |               |
| BARTLETT'S TEST OF<br>EQUAL VARIANCES |    | CHI-SQ  | DF     | P           |               |
|                                       |    | 0.19    | 2      | 0.9079      |               |
| CASES INCLUDED 9 MISSING CASES 0      |    |         |        |             |               |

The decision rules were applied:

F-test:  $f^* \leq F_{crit}$   $0.690 \leq 5.14$ , therefore do not reject the null  
P value:  $P \geq \alpha$   $0.534 \geq 0.05$ , therefore do not reject the null

A Tukey-pairwise comparison was initiated to compliment the one-way ANOVA results, as shown in Table M-3.

Table M-3  
Tukey-pairwise of O<sub>2</sub> Total Means for De-ionized H<sub>2</sub>O on Uncontaminated Soil  
(288 hr point)

| RUN | MEAN | HOMOGENEOUS<br>GROUPS |
|-----|------|-----------------------|
| 1   | 8264 | I                     |
| 2   | 8048 | I                     |
| 3   | 7881 | I                     |

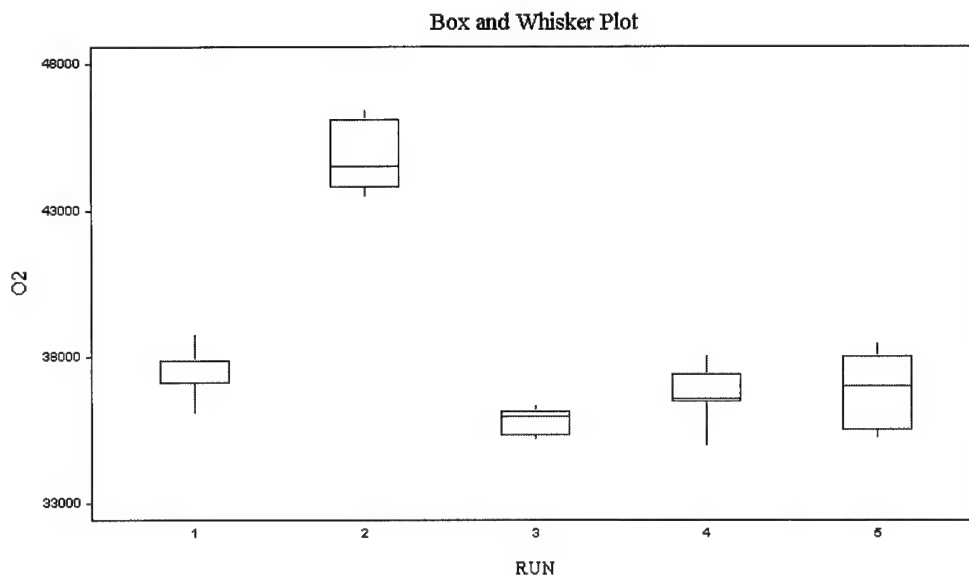
THERE ARE SIGNIFICANT PAIRWISE DIFFERENCES AMONG THE MEANS.

CRITICAL Q VALUE 4.469 REJECTION LEVEL 0.050  
STANDARD ERRORS AND CRITICAL VALUES OF DIFFERENCES VARY BETWEEN COMPARISONS BECAUSE OF UNEQUAL SAMPLE SIZES.

**STATISTIX<sup>®</sup> Results for PG<sub>1000</sub> on Uncontaminated Soil**

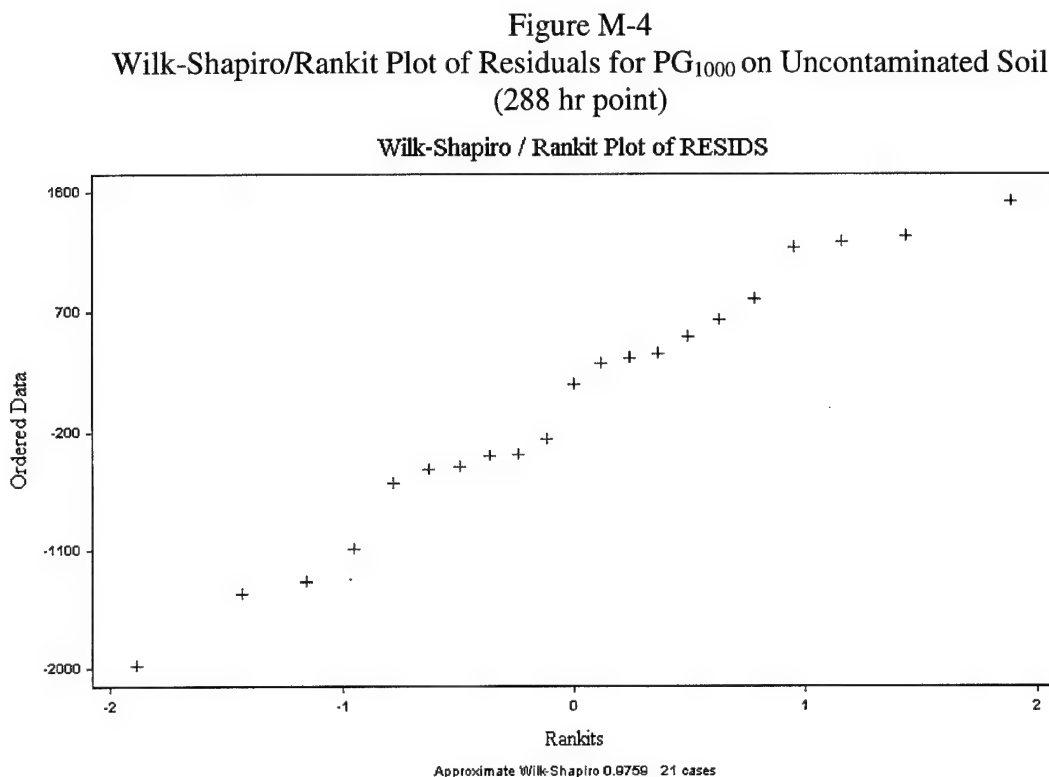
Outliers were checked on the data sets using a Box and Whisker plot as shown in Figure M-3.

Figure M-3  
Box and Whisker Plot of O<sub>2</sub> totals for PG<sub>1000</sub> on Uncontaminated Soil  
(288 hr point)



**Note:** No outliers were apparent in any of the respirometry runs (data sets).

The one-way ANOVA produced the residuals for the five different respirometry runs. The residuals were plotted using a Wilk-Shapiro/Rankit plot as shown in Figure M-4.



The residuals show aptness ( $R = 0.976$ ), thus statistical testing was continued with the one-way ANOVA results, as shown in Table M-4.

Table M-4  
One-way ANOVA Results for PG<sub>1000</sub> on Uncontaminated Soil  
(288 hr point)

| ONE-WAY AOV FOR O2 BY RUN         |        |           |           |       |        |
|-----------------------------------|--------|-----------|-----------|-------|--------|
| SOURCE                            | DF     | SS        | MS        | F     | P      |
| BETWEEN                           | 4      | 2.557E+08 | 6.392E+07 | 54.87 | 0.0000 |
| WITHIN                            | 16     | 1.864E+07 | 1164933   |       |        |
| TOTAL                             | 20     | 2.743E+08 |           |       |        |
|                                   |        |           |           |       |        |
| BARTLETT'S TEST OF                | CHI-SQ | DF        | P         |       |        |
| EQUAL VARIANCES                   | 5.13   | 4         | 0.2742    |       |        |
| CASES INCLUDED 21 MISSING CASES 0 |        |           |           |       |        |

The decision rules were applied:

|          |                     |   |
|----------|---------------------|---|
| F-test:  | $f^* \geq F_{crit}$ | $54.87 \geq 2.87$ , therefore reject the null |
| P value: | $P \leq \alpha$     | $0.00 \leq 0.05$ , therefore reject the null  |

A Tukey-pairwise comparison was initiated to determine which respirometry run means were not homogeneous, as shown in Table M-5.

Table M-5  
Tukey-pairwise of O<sub>2</sub> Total Means for PG<sub>1000</sub> on Uncontaminated Soil  
(288 hr point)

| RUN   | MEAN  | HOMOGENEOUS<br>GROUPS |
|-------|-------|-----------------------|
| ----- | ----- | -----                 |
| 2     | 44873 | I                     |
| 1     | 37551 | I                     |
| 5     | 37265 | I                     |
| 4     | 36837 | I                     |
| 3     | 35803 | I                     |

THERE ARE 2 GROUPS IN WHICH THE MEANS ARE  
NOT SIGNIFICANTLY DIFFERENT FROM ONE ANOTHER.

CRITICAL Q VALUE 4.469 REJECTION LEVEL 0.050  
STANDARD ERRORS AND CRITICAL VALUES OF DIFFERENCES  
VARY BETWEEN COMPARISONS BECAUSE OF UNEQUAL  
SAMPLE SIZES.

The results in Table M-2 and Table M-3 showed consistency from the respirometer, since the background soil treated with de-ionized water had mean O<sub>2</sub> consumption total that were consistent. The results of Table M-4 and Table M-5 revealed a significant difference in Run-2 compared to the other respirometry runs. This required Run-2 to be re-accomplishment.

**New Data: Means of Cumulative O<sub>2</sub> (μL) from Each Experimental Run**

Run-2 was re-accomplished and then replaced the old Run-2 data. The new data set is listed in Table M-6.

Table M-6  
Cumulative O<sub>2</sub> Consumption Totals (288 hr point)  
(Run-2, re-accomplished and included)

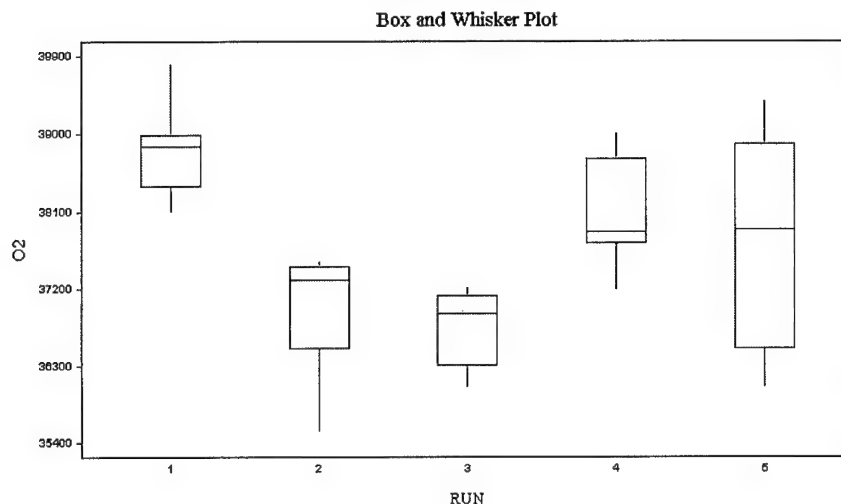
|           | PG <sub>1000</sub> on Uncontaminated Soil |       |       |       |       | Average | Std Dev |
|-----------|---|-------|-------|-------|-------|---------|---------|
| Run-1     | 37907                                     | 37092 | 36117 | 37865 | 38773 | 37551   | 998     |
| New Run-2 | 34871                                     | 36791 | 35905 | 36823 | 36634 | 36205   | 834     |
| Run-3     | 36319                                     | 35220 | 35318 | 36193 | 35963 | 35803   | 505     |
| Run-4     | 36455                                     | 37469 | 36587 |       |       | 36837   | 551     |
| Run-5     | 35282                                     | 38451 | 38062 |       |       | 37265   | 1729    |

**STATISTIX® Results for PG<sub>1000</sub> on Uncontaminated Soil (288 hr point)**

Outliers were checked on the data sets using a Box and Whisker plot as shown in Figure M-5.

Figure M-5

Box and Whisker Plot of O<sub>2</sub> totals for PG<sub>1000</sub> on Uncontaminated Soil  
(288 hr point)

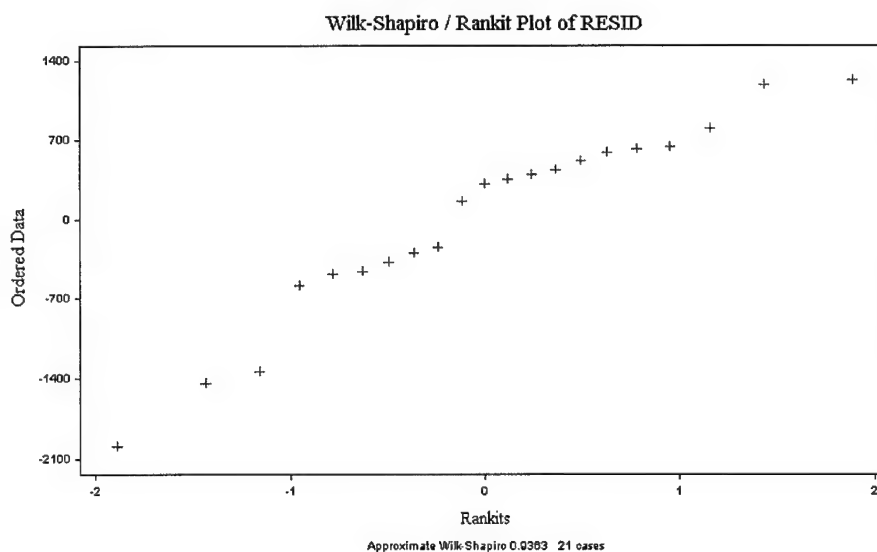


**Note:** No outliers were apparent in any of the respirometry runs (data sets).

The one-way ANOVA produced the residuals for the five different respirometry runs. The residuals were plotted using a Wilk-Shapiro/Rankit plot as shown in Figure M-6.

Figure M-6

Wilk-Shapiro/Rankit Plot of Residuals for PG<sub>1000</sub> on Uncontaminated Soil  
(288 hr point)



The residuals show aptness ( $R = 0.936$ ), thus statistical testing was continued with the one-way ANOVA results, as shown in Table M-7.



Table M-7  
One-way ANOVA Results for PG<sub>1000</sub> on Uncontaminated Soil  
(288 hr point)

| ONE-WAY AOV FOR O2 BY RUN             |    |           |         |        |        |
|---------------------------------------|----|-----------|---------|--------|--------|
| SOURCE                                | DF | SS        | MS      | F      | P      |
| BETWEEN                               | 4  | 9868719   | 2467180 | 2.75   | 0.0649 |
| WITHIN                                | 16 | 1.437E+07 | 897849  |        |        |
| TOTAL                                 | 20 | 2.423E+07 |         |        |        |
|                                       |    |           |         |        |        |
| BARTLETT'S TEST OF<br>EQUAL VARIANCES |    | CHI-SQ    | DF      | P      |        |
|                                       |    | 4.74      | 4       | 0.3147 |        |
| CASES INCLUDED 21 MISSING CASES 0     |    |           |         |        |        |

The decision rules were applied:

F-test:  $f^* \geq F_{crit}$   $2.75 \leq 2.87$ , therefore do not reject the null  
P value:  $P \leq \alpha$   $.065 \geq 0.05$ , therefore do not reject the null

A Tukey-pairwise comparison was produced (Table M-8) to confirm the one-way ANOVA results.

Table M-8  
Tukey-pairwise of O<sub>2</sub> Total Means for PG<sub>1000</sub> on Uncontaminated Soil  
O<sub>2</sub> Totals from Respirometry Runs (288 hr point)  
(Run-2, re-accomplished and included)

| RUN   | MEAN  | HOMOGENEOUS<br>GROUPS |
|---|-------|-----------------------|
| -----   | ----- | -----                 |
| 1   | 37551 | I                     |
| 5   | 37265 | I                     |
| 4   | 36837 | I                     |
| 2   | 36205 | I                     |
| 3   | 35803 | I                     |
| THERE ARE NO SIGNIFICANT PAIRWISE DIFFERENCES<br>AMONG THE MEANS. |       |                       |

The results of Table M-8 revealed that the respirometry runs were now homogenous.

## **Bibliography**

Alan R. Katritzky Research Group, Florida Center for Heterocyclic Chemistry Research, 1997

Amdur, M.O., J. Doull, and C.D. Klaassen, Cassarett and Doull's Toxicology: The Basic Science of Poisons. New York, Peragmon Press., 1991

Atlas, R. M., and R. Bartha, Microbial Ecology, Fundamentals and Applications, Third ed. The Benjamin/Cummings Publishing Company, Inc., 1993

Backer, D.S., D. Smith, and C.E. Habben, "Deicing Dilemma", Civil Engineering, Jul 1994, 56-59

Bausmith, D.S., Land Treatment of Aircraft Deicing Fluids, University of Pittsburgh Masters Thesis, 1997

Baker, J. A., Evaluation of the Natural Biodegradation of JP-8 in Various Soils using Respirometry. Air Force Institute of Technology Masters Thesis, 1995

Bridie, A.L., C.J.M. Wolf, and M. Winter, "BOD and COD of some Petrochemicals". Water Research. Vol 13, 627-630, 1979

Boluk, Y., and P. Levesque, Report On: Aircraft Deicing Fluid Recovery Concerns about the Quality of Recycled Glycol for use in Various Applications, Prepared for: Technology Department Union Carbide Canada Limited, September 10, 1990

Boyd, G.R., "Municipal Stormwater Permits: Developing Strategies for Compliance," Public Works; 61-66, Jan 1991

Brown, T.L., H.E. Lemay, Jr., and B.E. Bursten., Chemistry; The Central Science, Seventh ed, Prentice Hall, 1997

Cancilla, Devon .A., A. Holtkamp, L. Matassa, and X. Fang, Isolation and Characterization of Microtox® - Active Components From Aircraft De-Icing/Anti-Icing Fluids. Environmental Toxicology and Chemistry, Vol 16, No 3, 430-434, 1996

Cornell, J.S., Aspects of the Chemistry, Toxicity, and Biodegradability of Benzotriazole and its Derivatives. (Proposed Paper). Air Force Institute of Technology, University of Boulder Colorado, 1998

\_\_\_\_\_, D.A. Pillard, and M.T. Hernandez, Paper On: Chemical Components of Aircraft Deicer Fluid: How they affect Propylene Glycol Degradation rate and Deicing Waste Stream Toxicity, University of Boulder Colorado, Department of Civil, Environmental, and Architectural Engineering, 1997

Cox, D. P., "The Biodegradation of Polyethylene Glycols." Advanced Applied Microbiology, Vol 23, 173-194, 1978

Devore, J. L., Probability and Statistics for Engineering and the Sciences. Fourth ed, Duxbury Press, 1995

Dwyer, D.F., and J.M. Tiedie, Degradation of Ethylene Glycol and Polyethylene Glycol by Methogenic Consortia. Applied and Environmental Microbiology, Vol 46, No 1, 185-190, 1983

Eaton, A.D., L.S. Celsner, and A.E. Greenberg, Standards Methods for Examination of Water and Wastewater. 19th ed, American Public Health Association, Washington D.C., 1995

Ellis, T.G., B.F. Smets, and C.P. Leslie Grady Jr, Effect of Simultaneous Biodegradation of Multiple Substrates on the Extent Biodegradation Kinetics of Individual Substrates, Water Resources, Vol 70, No 27, 1998

Environmental Department of the Naval Facilities Engineering Service Center, Information Bulletin, <http://enviro.nfesc.navy.mil>, Mar 99

Fetter, C.W., Applied Hydrogeology, Third ed, Englewood Cliffs, NJ: Prentice-Hall, 1994.

Haines, J. R. and M. Alexander, "Microbial Degradation of Polyethylene Glycol". Applied Microbiology, Vol 29, No 5, 621 - 625, 1975

Hartwell, S.I., D.M. Jordahi, J.E. Evans, and E.B. May, Toxicity of Aircraft De-Icer and Anti-Icier Solutions to Aquatic Organisms, Environmental Technology and Chemistry, Vol 14, No 8, 1375 - 1286, 1995

HQ Air Force Center for Environmental Excellence (AFCEE), PRO-ACT Fact Sheet, TI 6159, July 1995.

Jank, B.E., H.M. Guo, and V.W. Cairns, Activated Sludge Treatment of Airport Wastewater Containing Aircraft De-icing Fluids. Water Research, Vol 5, 875 - 880, 1974

Johnson, L. M., Evaluation of the Natural Biodegradation of Aircraft Deicing Fluid Components in Soils. Air Force Institute of Technology Masters Thesis, 1997

Kaplan, D.L., J.L. Watsh, and A.R. Kaplan, Gas Chromatographic Analysis of Glycols to Determine Biodegradability. Environ.Sci. Technol, Vol 16, No10, 723-725, 1982

Kawai, F., T. Kimura, M. Fukaya, Y. Tani, K. Ogata, T. Ueno, and H. Fukami, Bacterial Oxidation of Polyethylene Glycol. Applied and Environmental Microbiology, Vol 35, No 4, 679 - 684, 1978

Kellner, D.K., Sorption of the Aircraft Deicing Fluid Component Methyl-benzotriazole in Soil. Air Force Institute of Technology Masters Thesis, 1999

Klecka, G.M., C.L. Carpenter, and B.D. Landenberger, Biodegradation of Aircraft Deicing Fluids in Soil at Low Temperatures. Ecotoxicology and Environmental Safety, Vol 25, 280 - 295, 1993

Leiter, J.L. and W.W. Funderbunk, Jr., "Everything You Always Wanted to Know about Storm Water Permit," Airport, Vol 3, Mar-Apr 1991

Lyman, E.A., Chapter 1-Handbook of Chemical Property Estimation Methods, Handbook of Chemical property Estimation Methods, McGraw-Hill, NY, 1982

MacDonald, D. D., I.D. Cuthbert, and P.M. Outridge, Canadian Environmental Quality Guidelines for Three Glycols used in Aircraft De-icing/Anti-icing Fluids. Report prepared for: EcoHealth Branch Environment Canada, 351 Boulevard St. Joseph, Hull Quebec, KIA 0H3, Sep 1992

Majewski, H.S., J.F. Kalverkamp, and D.P. Scott., Acute Lethality, and Sublethal Affects of Acetone, Ethanol and Propylene Glycol on the Cardiovascular and Respiratory Systems of Rainbow Trout (Salmo gairdneri). Water Resource, Vol 13, 217-221, 1978

Mallinckrodt Laboratory Chemicals, Material Safety Data Sheet for 1,2-Propandediol (Propylene Glycol), Mallinckrodt Laboratory Chemicals, 1997

Massaccesi, M., Indirect UV Detection in HPLC Determination of UV-Transparent Non-Electrolytes in Pharmaceutical Dosage Forms. IL Farmaco, Vol 47, 753-767, 1992

McGahey, C. and E.J. Bouwer. "Biodegradation of Ethylene Glycol in Simulated Subsurface Environments". Water Science Technology. 26, 41-49, 1992

McKane, L., and J. Kandel, Microbiology, Essential and Applications, Second ed; McGraw-Hill, Inc., 1996

Mericas, D., and B. Wagoner, "Balancing Safety". Water Environment and Technology, Water Environment and Technology, Vol 6, 39 -43, 1994

Metcalf and Eddy, Inc., Wastewater Engineering-Treatment, Disposal, and Reuse, Third ed, McGraw-Hill, Inc., 1991

Metting, F. Blaine. Jr, "Structure and Physiological Ecology of Soil Microbial Communities," in Soil Microbial Ecology: Applications in Agricultural and Environmental Management. Ed, F. Blaine. Metting, Jr.,NY; Marcel Dekker Inc., 1993

Micro-Oxymax x6.03 Instruction Manual. Publication number 0125-5002. Columbus, Ohio: Columbus Instruments International Corporation, 1996

Miller, L.M., Investigation of Selected Potential Environmental Contaminants; Ethylene Glycol, Propylene Glycols, and Butylene Glycols. NTIS Report PB80-109110, Franklin Research Center, Philadelphia, Pennsylvania, 1979

Mills, A.L., Metal Requirements and Tolerances, Reference found in *Manual of Environmental Microbiology*, Hurst, C.J., G.R. Knudsen, M.J. Melnerney, L.D. Stetzenbach, M.V. Walter, American Society for Microbiology, 1996

National Safety Council (NSC), Environmental Health Center, Division of the National Safety Council, 1025 Connecticut Avenue, NW, Suite 1200, Washington, DC 20036, Information Bulletin, <http://www.nsc.org/index.htm>, Jan 1999

Nitschke, L, and L. Huber, Determination of Glycols by HPLC with Refractive Index Detection. Fresenius J Anal Chem. Vol 349, 451-453, 1994

\_\_\_\_\_, H. Wagner, G. Metzner, A. Wilk, and L. Huber, Biological Treatment of Waste Water Containing Glycols From De-Icing Agents. Water Resources, Vol 30, No 3, 644 - 648, 1996

Oakley, M.M., and C.L. Forrest, "The Clock is Ticking to Comply with New Stormwater Regulations". Water Environment and Technology, 51 - 56, Mar 1991

O'Malley, A.H., Biodegradation of Deicing Agents in Various Soil Types. Air Force Institute of Technology Masters Thesis, 1997

PMC Specialties Group, "COBRATEC (R) TT-100 Technical Bulletin." Technical Bulletin COR4317. PMC Specialties Group, Inc., 1996

\_\_\_\_\_, Material Safety Data Sheet for COBRATEC (R) TT-100, PMC Specialties Group, Inc., 1996

Raja, L.M., G. Elmaivaluthy, R. Palaniapan, and R.M. Kirshnan, Novel Biotreatment Procedures for Glycol Waters. Applied Biochemistry and Biotechnology, Vol 28/29; 827-841, 1991

Razo-Flores, E., M. Luijten, B. A. Donolon, G. Lettinga, and J. A. Field, Complete Biodegradation of the Azo Dye Azodisalicylate under Anaerobic Conditions. Environmental Science and Technology, Vol 31, 2098-2103, 1997

Rice, P. J., T. A. Anderson, J. R. Coats, "Evaluation of the Use of Vegetation for Reducing the Environmental Impact of Deicing Agents." Phytoremediation of Soil and Water Contaminants, Eds. E. L. Kruger, T. A. Anderson, J. R. Coats, ACS Press, Washington DC. 1997

Safferman, S. L., G. S. Sirvalure, and L.E. Foppe, Deicing Fluid Treatment in Batch-Loaded Aerobic Fluidized Bed Reactor, Journal of Environmental Engineering, Vol 124, No. 1, 11-15,

Sawyer, C.N., P.L. McCarty, and G.E. Parkin, Chemistry for Environmental Engineering, Fourth ed; McGraw-Hill, Inc., 1994

Sax, N.I., and R.J. Lewis, Dangerous Properties of Industrial Materials. Seventh Ed, Van Nostrand Reinhold, New York, 1989

Schwarzenbach, R. P., P. M. Gschwend, and D. M. Imbroden, Environmental Organic Chemistry, First ed, Wiley-Interscience, NY, 1993

Shupack, D. P., Influence of Vegetation on Mineralization of Propylene Glycol in Soil: Reducing Environmental Impact of De-icing Fluids in Runoff Water. Clemson University Masters Thesis, 1997

Sills, R. D., and P. A. Blakeslee, "The Environmental Impact of Deicers in Stormwater Runoff": Michigan Department of Natural Resources - Environmental Impact Study, Lansing: Surface Water Quality Division, 323-340, March 1991

Society of Automotive Engineers (SAE), "Specification for De-icing/Anti-icing Fluid, Aircraft, SAE Type 1," AMS 1424, Warrendale PA, 1992

Strong-Gunderson, J. M., S. Wheelis, S. L. Carroll, M. D. Waltz, and A.V. Palumbo, Degradation of High Concentrations of Glycols, Antifreeze, and Deicing Fluids, Microbial Processes for Bioremediation. Columbus, Battel Press. Vol 3, 265-270, 1995

Thomas, J. D., Surfactant Enhanced Microbial degradation of JP-8 Contaminated Soil. Air Force Institute of Technology Masters Thesis, 1996

Totten, C.T., Use of Respirometry to Determine the Effect of Nutrient Enhancement on JP-8 Biodegradation. Air Force Institute of Technology Masters Thesis, 1995

Transport Canada, State-of-the-Art Report of Deicing/Anti-icing. Ottawa, Ontario, Canada, Ministry of the Environment, Professional and Technical Services, Facilities and Environmental Management, 1985

Weber, E. J., and R. A. Larson, Reaction Mechanisms in Environmental Organic Chemistry, Lewis Publishers, Boca Raton, 1994

Whitman, B.W., D.C. Coleman, and W.J. Wiche, Prokaryotes: The Unseen Majority, Proc. National Acad Sci. USA, Vol. 95, pp. 6578 - 6583, 1998

Wijk, A., and B. Karlberg, Determination of Glycol in Aircraft Ground Deicing/Anti-Icing Fluids Flow Injection with Refractive Index Detection, Talanta, Vol 41, No 2, 395 - 400, 1994

## Vita

Captain Baron W. Burke was born on June 30, 1969 in Seattle, Washington. He graduated from Seattle Lutheran High School in 1987, and attended South Seattle Community College where he earned an Associate of Arts degree in December 1990. In concurrence he attended the University of Washington and graduated with a Bachelor of Science degree in Civil Engineering in June 1993. He was commissioned as a 2<sup>nd</sup> Lieutenant in the U.S. Air Force on June 11, 1993. He entered active duty service on December 1, 1993 working as a base civil engineering officer at the 92<sup>nd</sup> Civil Engineering Squadron, Fairchild A.F.B., Spokane, Washington. From May 1995 to August 1995, he was sent on temporary duty to Soto Cano Air Base, Honduras as a base civil engineering officer. From July 1996 to September 1996, he was TDY at Dhahran A.B., Saudi Arabia, as a base civil engineering and programming officer. He was subsequently selected to study for his Masters of Science in Engineering and Environmental Management at the Air Force Institute of Technology from July 1997 to March 1999. Upon completion of the AFIT program, Captain Burke will be assigned to 366<sup>th</sup> Civil Engineering Squadron at Mountain Home A.F.B., Idaho.



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| 13. ABSTRACT (Maximum 200 words)<br>Aircraft de-icing fluids (ADFs) are used worldwide to ensure safe aircraft operations. This research effort was conducted to analyze the biodegradation effects of two chemical components of ADFs, propylene glycol (PG) and tolyltriazole (TTA), in a high-clay soil. The research used four test methods; automated respirometry, high performance liquid chromatography (HPLC), microbial colony population counts (MCPC), and agar well diffusion tests (AWDT). The research was partitioned into two phases of investigation. Phase-one analyzed individual and combined ADF chemical components on uncontaminated soil. The presence of TTA, from 25 - 1,000 mg/kg, reduced the maximum respiration rate of 1,000 mg/kg PG alone; however, cumulative respiration over the two-week study period was proportionality higher for TTA (25 - 500 mg/kg). Rates and respiration totals for soil exposed to TTA (25 - 750 mg/kg) alone, were not significantly different from background soil; however, rate and respiration totals for PG (1,000 mg/kg) alone were significantly higher. The HPLC percentage of recovered TTA, with or without PG presence, indicated a loss (biodegradation and/or absorption) of TTA within the soil. Kellner (1999) conducted HPLC for absorption/desorption of TTA on the same (high-clay) soil. MCPC and AWDT indicated no measurable toxic effects to microbial populations/health occurred from ADF chemical components. Phase-two research conducted reapplication of ADF chemicals on acclimated soils from phase-one. Initial respiration rates from application of 1,000 mg/kg PG on acclimated soil (PG 1,000 mg/kg) compared to 1,000 mg/kg PG on uncontaminated soil. The acclimated soil produced a significantly larger initial rate of respiration. |  |   |                                    |  |
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